SCIENTIFIC MEMOIRS

BY

MEDICAL OFFICERS OF THE ARMY OF INDIA.

EDITED BY

SURGEON MAJOR-GENERAL J. CLEGHORN, M.D.,
SURGEON-GENERAL WITH THE GOVERNMENT OF INDIA.

PART IX.

1895.

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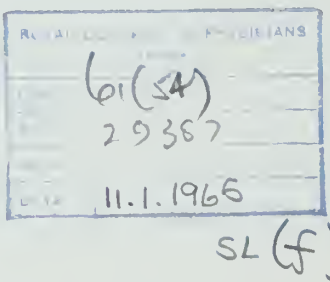


The Physiological action of Snake-venom.

BY

BRIGADE-SURGEON-LIEUTENANT-COLONEL D. D. CUNNINGHAM,

C. I. E., F. R. S.

IN the course of a prolonged series of experiments, which were primarily undertaken with a view of testing the efficacy of various reputed remedies for cases of snake-bite, certain phenomena have presented themselves, appearing to justify some conclusions in regard to the nature and physiological action of the venom of Colubrine and Viperine snakes, which appear to be of sufficient interest to merit publication, and it is accordingly proposed to draw attention to them in the following pages. Certain of the conclusions are by no means new; but they are, at the same time, not in accord with currently-accepted opinion, and, therefore, appear to be worthy of renewed discussion in the light of newly-recorded facts, and, in any case, they have not been arrived at hastily, but as the result of much experimental work.

Any course of experiments on ordinarily reputed remedies for cases of snake-bite is somewhat heartless work from the monotonously futile results which it yields when regarded solely in the light of a quest for practical means of contending with the action of snake-venom; but it possesses one great advantage in enforcing the constant employment of minimal lethal doses of the latter and, therefore, in affording opportunities for the accurate study of the precise sequence of events occurring in the course of the intoxication, which are not present in cases where relatively large doses enter the system, and where, consequently, the progress of the symptoms is much more accelerated.

Various factors would appear to have co-operated to give rise to misconceptions in regard to the precise nature and the physiological action of snake-venoms. The employment of excessive doses has, in many cases, served to obscure details in the course of the intoxication by hurrying on the symptoms so rapidly that their sequence could not be accurately followed; confusion has arisen from comparing the results of the action of the venom in different cases in which no precautions had been taken to secure any accurate parallelism in the proportions of the doses to the weights of the recipient animals; the use of fresh venom, and specially its introduction in the natural fashion by means of bites, has, probably in some instances, led to the effects of the specific toxins being confounded with those of pathogenic organisms which may enter the system along with them; and, finally, in so far as Viperine venom is concerned, errors have

crept in from a failure to recognise and discriminate the presence and action of two distinct toxic principles.

As it has been possible to study the action of the venom of the cobra (*Naia tripudians, Merr.*) and of Russell's viper (*Vipera russellii, Strauch*) most closely, and as these snakes may fairly be taken as the most lethal representatives of the families of Ophidia to which they belong, the data and conclusions regarding them naturally form the first subject for discussion, leaving the question of the extent to which similar conclusions are applicable in respect to the poisons of other venomous Colubrine and Viperine snakes for subsequent consideration.

I.—The Physiological action of the venom of *Naia tripudians.*

Almost all the observers—who, like Fayrer and Brunton, the Members of the Indian Commission, Wall, Aron and Calmette, have specially studied the physiological action of cobra-venom within recent years—seem to assume that it is necessarily a primary neurotic poison, acting directly upon the nervous, and specially the central nervous apparatus to give rise to the characteristic symptoms of the intoxication, and finally to cause death by asphyxia dependent on a paralytic suppression of respiratory movements. When, however, we come to examine the grounds on which this belief is founded, they certainly do not appear to possess any conclusive value, consisting, as they do, of observations showing that the blood, although of extremely dark colour when first removed from the dead bodies of poisoned animals, ordinarily rapidly brightens on exposure to air, and of the fact that nervous phenomena constitute conspicuous symptoms during the progress of the intoxication. Now no one can deny either of these data; but, at the same time, the question remains whether they warrant the conclusions which have been derived from them.

Taking them in order we have, in the first place, to consider the question of the change of colour ordinarily occurring in the blood of poisoned animals on exposure to air. It appears to be assumed that this is conclusive evidence that no loss of respiratory property can have taken place, and that, therefore, the asphyxia, which is the immediate cause of death, is solely dependent on failure of respiratory movements incident on a directly paralytic action of the venom on the nervous centres. As a matter of fact, however, the evidence is by no means conclusive, as it is by no means necessary that the blood should wholly lose its respiratory capacity in order to the occurrence of fatal asphyxia. This point has been carefully worked out in connection with poisoning by Carbonic oxide (CO), and it has been clearly shown that, in order to induce a fatal result, it is only necessary that a loss of 30 per cent. should occur in the respiratory capacity of the blood, *i.e.*, that the capacity of absorbing oxygen should be diminished by 30 per cent. But, if this be so, it is clear that blood, which has undergone suf-

ficient loss in respiratory property to cause death, may still be liable to undergo very conspicuous changes in colour according to the degree to which it is deprived of, or exposed to, the influence of atmospheric oxygen, and, as a matter of fact, we find such changes occurring in the case of CO-blood. For example, in the case of a fowl, which had just been poisoned by exposure to an atmosphere highly charged with CO, the blood when first removed from the heart was of a peculiar, brick-red colour, very similar to that which is so frequently present in the blood in cases of poisoning by Daboia-venom, but, after a brief exposure to the air, the surface became of an extraordinarily vivid scarlet. The original colour of the blood in this case must have been dependent on the mixture of that of CO-hæmoglobin with that of reduced hæmoglobin, whilst that which it subsequently acquired was the result of a mixture of CO-hæmoglobin with oxyhæmoglobin. The alteration in colour could not have been connected with any change in the relatively stable CO-hæmoglobin, but must have been dependent on oxidation of the residual hæmoglobin which had undergone general reduction in connection with the antecedent failure and final abolition of respiratory movements.

Now there can be no question that in cases of CO-poisoning we have to deal with asphyxia, primarily induced by loss of respiratory property in the blood, and yet conspicuous changes in the colour of the latter are liable to attend exposure to atmospheric oxygen. The changes in the blood primarily caused by the CO imply diminished respiratory property, and this in its turn finally leads to diminution and cessation of respiratory movements and thus to a cessation of exposure of the blood to the air, and with this, a secondary factor of blood-change comes into play. The CO reduces the respiratory property by replacing unstable oxyhæmoglobin by relatively stable CO-hæmoglobin, and the ultimate result of this is to occasion diminution and finally cessation of the exposure of the yet unaltered hæmoglobin to the atmospheric air, and therefore to its reduction.

It is clear, then, that the change in colour which the blood in cases of death from cobra-venom ordinarily undergoes on exposure to air is no satisfactory and conclusive evidence that the poison has not occasioned alterations in respiratory capacity in the fluid of sufficient magnitude to give rise to fatal results. All that the phenomenon does show is that a total loss of respiratory property has not occurred when death takes place ; but, as we have just seen, it is quite unnecessary that such a loss should happen in order to induce death. It will be presently shown that cobra-venom does produce most conspicuous effects on the respiratory property of blood ; but, in the meantime, assuming that it may do so, and knowing that a reduction of 30 per cent. in respiratory property is sufficient to cause death by asphyxia, the colour of the blood after death in ordinary cases of cobra-poisoning may be taken to be dependent on the presence

of two-thirds of blood containing reduced hæmoglobin with one-third of blood the respiratory properties of which have been abolished without the development of any bright red colouring matter like that of CO-hæmoglobin ; and, if this be so, it is quite clear that the exposure of the two-thirds of reduced quality to the influence of atmospheric oxygen must be followed by conspicuous changes in the colour of the fluid. As will be presently shown one action of the venom on the blood is to give rise to intense and permanent darkening in the colour of the latter, and the mixture of blood which has undergone such change with blood of highly reduced quality must imply the presence of very dark coloration, but of a coloration liable to be very considerably brightened on exposure to air in consequence of absorption of oxygen by the hæmoglobin. Under ordinary circumstances, then, we should naturally expect that such a change of colour should occur even if the venom have the power of reducing the respiratory property of the blood to a fatal extent, because, under ordinary circumstances, the amount of venom which enters the blood is relatively small, and the entrance is only gradually effected by absorption; but, as will presently be shown, no such change takes place when death follows the direct introduction of relatively large quantities of venom into the circulation. In the one case, death takes place long before the respiratory property of the blood has been entirely abolished, and consequently exposure to air is followed by change of colour; in the other, the direct introduction of a large quantity of venom determines immediate and total loss of respiratory property, and with this loss of capacity for change of colour under the influence of atmospheric oxygen. Taking all the facts into consideration, it is clear that they are of such a nature as to deprive the first argument for the belief in the primarily neurotic action of cobra-venom of all conclusive value.

On proceeding to consider the second foundation on which this belief rests, it can, I think, be shown to be of an equally unsatisfactory nature, and here again the data regarding poisoning by CO come in to afford very valuable information, because they show that nervous symptoms of a nature very closely similar to those characteristic of intoxication by cobra-venom may be developed in connection with the establishment of decreased respiratory property in the blood. On referring to the most recent works on physiology and legal medicine, we find that the characteristic symptoms attending intoxication by CO are of the following nature : * The initial stage is marked by increased lachrymal, salivary and nasal secretion, headache, giddiness, accelerated respiration, and occasionally nausea and vomiting. This is followed by the paralytic stage with staggering gait and paralysis, especially of the lower or hinder extremities. Finally

* 1. " Ueber die Kohlenoxyd vergiftung vom medicinal und sanitäts polizeilichen Standpunkte." Von Dr. Med. Robert Stoermer, Arzt in Berlin. Vierteljahrschrift für gerichtliche Medecin. Jahrgang, 1895, 2, Heft s. 367.
2. Lehrbuch der gerichtlichen Medicin. Dr. EduardR. von Hofmann. Wien u. Leipzig, 1893.

comes the stage of asphyxia with infrequent, primarily deep, subsequently brief gasping respirations, anæsthesia, coma, and death, which, in acute cases, is preceded by violent general convulsions, a phenomenon which is generally absent in chronic cases. Turning now to the symptoms attending intoxication by cobra-venom, we again find a preliminary acceleration of respiratory movement, increased nasal, lachrymal and salivary secretion, nausea and vomiting, paralysis, especially of the lower or hinder extremities, slow and shallow respiration, coma and death, preceded by general convulsions in acute cases and ordinarily occurring without them in chronic ones. But in the case of poisoning by CO, there appear to be no good grounds for doubting that the primary and essential action of the gas is to induce a gradually progressive diminution in the respiratory property of the blood owing to the gradual substitution of CO-hæmoglobin for oxyhæmoglobin, and that the nervous symptoms are dependent on this and not on any direct action on the nervous apparatus. But, if this be so, it is clear that in the case of poisoning by cobra-venom the parallel series of nervous phenomena which are present may have a like origin, and that their occurrence affords no conclusive evidence that the toxin possesses any direct neurotic properties. The mere presence of nervous symptoms does not prove that they are dependent on any direct action of the venom on the nervous apparatus, and the majority of those which are present are such as can be unequivocally shown to arise in connection with progressive loss in the respiratory property of the blood, so that the evidence in place of giving any decisive support to the theory of a direct and primary neurotic action, is rather in favour of that of primary blood change.

But it is fortunately unnecessary to rest satisfied with such theoretical considerations, as it can be readily demonstrated that cobra-venom does conspicuously affect the respiratory properties of the blood both within the body and outside. It was long ago pointed out by Boag that the mixture of relatively large quantities of snake-venom with blood produces an immediate effect on its colour and coagulability, but, as Richards justly remarks, the value of this observation as evidence for the occurrence of primary blood-change as the essential cause of death in cases of poisoning by cobra-venom is minimised by the fact that in the latter the proportion of venom to the mass of blood with which it mingles is always very small and ordinarily excessively so. The average quantity of dried material to be obtained from the venom ejected by a cobra at any one time is only about 0·254 gramme, but this is amply sufficient to secure the death of a man of 70 kilos in weight and whose blood probably amounts to between 4,000 and 5,000 grammes, or 16,000 to 20,000 times the weight of the dried venom. The results of the following series of experiments, however, in some of which only fractional quantities of venom acted upon relatively large masses of blood, clearly show that the potency of the former as a factor of blood change is such that the amounts which are liable to be introduced by a single

bite are quite sufficient to affect masses of blood of sufficient magnitude to account for the death of even very large animals in consequence of the bites of cobras.

Experiment I.—On the action of relatively large quantities of cobra-venom on blood outside the body :—

Two sterilised test tubes, *a* and *b*, were taken, and 1 c.c. of distilled water introduced into each of them.

The water in *a* was unmixed with anything, but that in *b* contained 0·01 gramme of dried venom, of which 0·0005 gramme constituted a minimal lethal dose for fowls weighing about 1 kilo.

A little blood from a fowl which had just been killed was then introduced into each tube.

The blood in *a* exhibited the normal phenomenon of very rapid coagulation and subsequent brightening of the surface of the coagulum to a vivid scarlet; that in *b*, immediately assumed an intensely dark, almost black colour, and remained permanently perfectly fluid.

Experiment II.—Action of 0·05 gramme of dried venom on 11·5 grammes of blood :—

A solution of 0·05 gramme of dried venom in 0·5 c.c. of distilled water was introduced into a test tube and 11·5 grammes of blood added to it direct from the throat of a fowl and thoroughly mixed by shaking.

The blood assumed a black colour at once and never shewed any tendency to coagulate.

Half an hour later the peripheral portions of the red blood-corpuscles were practically entirely dissolved, leaving the nuclei free in a dark red fluid.

Experiment III.—Action of 0·01 gramme of dried venom on 16·7 grammes of blood :—

This experiment was conducted in precisely the same fashion as the previous one.

The blood coagulated rapidly. At first it was of a deep plum-colour, but rapidly began to darken, and half an hour after the beginning of the experiment was black throughout. No serum ever separated from the soft, gelatinous clot.

One hour after the beginning of the experiment the peripheral portions of the red corpuscles had undergone almost as complete solution as in experiment *II.*

Experiment IV.—Action of 0·01 gramme of dried venom on 26·2 grammes of blood outside the body :—

Two covered, sterilised glass capsules, *a* and *b*, were taken; into the former 1 c.c. of distilled water containing 0·01 gramme of dried venom in solution, and into the latter 1 c.c. of pure distilled water were introduced.

At 10-32 A.M., 26·2 grammes of blood were received into *a*, and 24·1 grammes into *b*, direct from the throat of a fowl.

a. The blood coagulated at once.

11 A.M.—Surface of the coagulum dull, dark red.

11-35 A.M.—Surface considerably darker than before.

12 Noon.—Surface of the coagulum almost black.

12-30 P.M.—No contraction of the coagulum from the sides of the capsule. Only a very little free serum present, and that opaque and dark red.

On the following day, at 9·55 A.M., the coagulum was black throughout. There was no separation of it from the sides of vessel to which it adhered firmly. Only about 1 c.c. of free serum was present; opaque, intense brownish red and yielding an abundant whitish precipitate on dilution with distilled water.

b. The blood coagulated at once.

11 A.M.—Surface of the coagulum bright scarlet.

12-30 P.M.—Surface vivid scarlet; coagulum already well contracted from the sides of the vessel; serum limpid, straw coloured.

On the following morning the substance of the coagulum was plum-coloured, and the surface dull scarlet; coagulum floating free in 8·75 c.c. of deep red serum.

Experiment V.—Action of 0·002 gramme of venom on 27·1 grammes of blood outside the body :—

Two capsules, prepared as in the previous experiment *a*, containing 0·002 gramme of dried venom in 1 c.c. of distilled water, and *b*, 1 c.c. of distilled water alone.

At 10·40 A.M. 27·1 grammes of blood were introduced into *a*, and 18·26 grammes into *b*, from the throat of a fowl.

a. The blood coagulated at once.

11-5 A.M.—Exudation of serum beginning to occur; surface of clot scarlet, but not so vivid in colour as in *b*.

1-30 P.M.—Surface of clot very deep, dull red.

On the following morning the coagulum throughout was almost black; there was about 6·5 c.c. of free serum of very deep, blackish-red colour, quite opaque, and throwing down an abundant whitish precipitate on dilution with distilled water.

b. Coagulation occurred at once.

11-5 A.M.—Exudation of serum beginning to occur; surface vivid scarlet.

1-30 P.M.—Surface of coagulum vivid scarlet.

On the following morning the surface of the coagulum was bright scarlet, and the substance plum-colour, and about 4 c.c. of bright red serum were present.

Experiment VI.—Action of 0·002 gramme of dried venom on 29·9 grammes of blood :—

Two capsules, *a* and *b*, prepared as in the previous experiments, the former containing 1 c.c. of distilled water with 0·002 gramme of dried venom in solution, the latter 1 c.c. of distilled water alone.

At 10·46 A.M., 29·9 grammes of blood from the throat of a fowl were introduced into *a*, and 29·1 grammes into *b*, and in both cases the vessel was shaken so as to mix the blood and water thoroughly.

a. The blood coagulated rapidly. At first the surface of the coagulum was of a deep red, but in place of brightening on exposure to air, it speedily darkened.

10-55 A.M.—Coagulum almost black throughout.

2 P.M.—The coagulum everywhere in contact with the sides of the vessel, and not a trace of free serum visible.

On the following morning the surface of the clot was almost black and its substance black throughout; it was densely adherent to the sides of the vessel, and, when turned out of it, its lateral surfaces underwent no change in colour on exposure to air. There was not a trace of any free serum present. The peripheral portions of the red corpuscles were entirely dissolved and the nuclei free.

b. The blood coagulated rapidly. The surface of the clot was at first dark red, but speedily became bright scarlet.

2 P.M.—Surface bright scarlet and substance plum-coloured; clear straw coloured serum exuding.

On the following morning the surface was dull scarlet and the substance plum-coloured. The coagulum was readily detached from the sides of the vessel and its lateral surfaces brightened to dull scarlet on exposure to air, 6·5 c.c. of red serum were present, and the red corpuscles were well preserved.

Experiment VII.—Action of 0·002 gramme of dried venom on 26·4 grammes of blood:—

This experiment was conducted in precisely the same fashion as the preceding one, *a* receiving 26·4, and *b*, 22·6 grammes of blood from the neck of a fowl at 10-32 A.M.

a. The blood remained fluid for about eight minutes, and then slowly coagulated. It was at first dark coloured and for a short time the surface brightened considerably on exposure to air. Renewed darkening, however, soon set in, and progressed rapidly.

10-52 A.M.—Surface dark red; substance of the clot plum-coloured.

1 P.M.—Practically black throughout; not a trace of free serum.

On the following morning the coagulum was black throughout. It was very firmly adherent to the sides of the vessel, and, when turned out of it, its lateral surfaces underwent no change of colour on exposure to air. There was not a trace of any free serum, and the nuclei alone of the red corpuscles were recognisable.

b. The blood was at first dark coloured, but the surface of it rapidly became vivid scarlet.

11-10 A.M.—Surface bright scarlet; serum beginning to exude.

1 P.M.—Surface vivid scarlet; substance plum-coloured; exudation of serum advancing.

On the following morning the surface of the coagulum was dull scarlet and the substance plum-coloured. When turned out of the vessel the edges of the clot became dull scarlet. The bodies of the red corpuscles were well preserved and 3·5 c.c. of limpid bright red serum were present.

Experiment VIII.—Action of 0·0005 gramme of dried venom on 26·3 grammes of blood:—

This experiment was conducted in precisely the same fashion as the preceding two, save that *a* contained only 0·0005 gramme of dried venom.

The blood was introduced into the capsules at 10-58 A.M., 26·3 grammes being introduced into *a*, and 30·46 grammes into *b*.

a. Coagulation occurred within four minutes. The surface of the clot was at first dark red. It gradually brightened to scarlet, and then presently began to darken anew.

11·30 A.M.—Surface dark red; substance plum-coloured.

12 Noon.—Surface almost black; substance plum-coloured; no sign of any exudation of serum.

1 P.M.—Surface and substance nearly black; no visible contraction of the clot, but a little dark-red serum present.

On the following morning the coagulum was practically black throughout. There was no appreciable retraction of the clot from the sides of the vessel, but about 0·25 c.c. of very dark opaque serum, depositing an abundant whitish precipitate on dilution with distilled water, was present. The sides and artificially exposed surfaces of the clot showed no change of colour on exposure to air. The peripheral portions of most of the red corpuscles were absent, leaving the nuclei free. The texture of the coagulum was soft and gelatinous.

b. The blood coagulated rapidly, and the surface of the clot soon became vivid scarlet.

11-30 A.M.—Surface vivid scarlet; substance dark red; exudation of serum just beginning.

12 Noon.—Surface bright scarlet; substance dull red; exudation of serum advancing.

1 P.M.—Surface vivid scarlet; substance plum-coloured; clot well contracted from the sides of the vessel, and a large quantity of clear, straw-coloured serum present.

On the following morning the surface of the clot was dull scarlet and the substance of it plum-coloured. The coagulum was widely shrunk from the sides of the vessel and lying free in 9 c.c. of clear, straw-coloured serum. When the coagulum was removed from the serum it rapidly became bright scarlet on the surface and dull red on the sides, and surfaces artificially exposed by section also became scarlet on exposure to air. The texture of the clot was firm and the red corpuscles were well preserved.

In the above experiments the mixture of the venom and the blood was

effected outside the body, but in the following ones it took place within the body as in cases of snake-bite.

Experiment IX.—Effects produced on the blood of a fowl by the hypodermic injection of 0·01 gramme of dried venom :—

a. A fowl received an injection of 0·01 gramme, *i.e.*, twenty times a minimal lethal dose, of dried venom in 1 c.c. of distilled water at 10-38 A.M. At 11-20 A.M., asphyxial convulsions had set in, and 55·5 c.c. of blood were received from the throat in a sterilised and covered glass capsule.

b. At 11-22 A.M., 39·5 c.c. of blood from the throat of a healthy fowl were received in a similar capsule.

a. The blood was of a dull, plum-red colour and coaguluted at once.

11-45 A.M.—Dull plum-coloured, the surface somewhat brighter than the substance of the coagulum. (The capsule was now placed in an incubator at 37°C., and the cover replaced by a bell-glass.)

12-3 P. M.—Exudation of serum beginning to occur.

12-32 P.M.—Surface very dark plum-coloured throughout.

2-5 P.M.—Serum deep, brownish-red ; coagulum very dark plum-coloured throughout.

On the following morning the surface of the coagulum was of a very dark brown and the substance deep brownish plum-coloured. The coagulum was not in the least retracted from the sides of the vessel, but adhered firmly to them. The free serum, which amounted to 5·5 c.c., was of an intensely dark brown colour.

b. The blood coagulated at once and the surface rapidly became of a bright scarlet colour.

11-45 A.M.—Surface bright scarlet and substance dull red. (The capsule was now placed in the incubator like that of *a.*)

12-3 P.M.—Exudation of serum beginning to occur.

12-32 P.M.—Surface of the coagulum bright scarlet ; substance dull red.

2-5 P.M.—Surface bright scarlet ; substance plum-coloured ; serum straw-coloured.

On the following morning the surface of the coagulum, where covered by serum, was plum-coloured, and, where exposed to the air, dull scarlet ; the clot was well retracted from the sides of the capsule and non-adherent. There were 4·5 c.c. of dull reddish serum present.

Experiment X.—Effects produced on the blood of a fowl by the hypodermic injection of 0·005 gramme, i. e., *ten times a minimal lethal dose of dried venom :—*

a. A fowl received an injection of 0·005 gramme of dried venom in 1 c.c. of distilled water at 8-15 A.M. Two hours later it was dying and 19 c.c. of blood from its throat were received in a sterilised glass capsule.

b. At 10-35 A.M., 26·5 c.c. of blood from the throat of a healthy fowl were received into a similar capsule.

a. The blood was of a very dark colour and coagulated at once. The surface of the coagulum gradually brightened to scarlet.

10-30 A.M.—Exudation of serum beginning to occur.

11-20 A.M.—One or two pools of serum on the surface.

11-45 A.M.—Slight retraction of the clot from one point of the sides of the capsule.

1-20 P.M.—Hardly any retraction of the clot from the sides of the vessel. Surface very dark red, save in the centre.

On the following morning the coagulum was of a very deep brownish red throughout. No farther retraction from the sides of the capsule had occurred. The serum was of a deep brownish red and opaque and amounted to 3 c. c.

b. The blood was originally of a very dark red. It coagulated at once, and the surface of the clot rapidly assumed a vivid scarlet colour.

11-20 A.M.—An abundance of free serum present; surface of clot bright scarlet.

11-45 A.M.—Coagulum well retracted from the sides of the capsule.

1-20 P.M.—Coagulum widely retracted from the sides of the capsule; surface bright scarlet throughout; serum limpid, straw-coloured.

On the following morning the surface of the coagulum, where covered by serum, was deep plum-coloured, and, where exposed to the air, dull scarlet. The serum was limpid, bright red and amounted to 11·0 c.c.

Experiment XI.—Effects produced on the blood of a fowl by the introduction of 0·34 gramme of dried venom directly into the circulation:—

A fowl, weighing 1550 grammes, received an injection of 0·34 gramme of dried venom, of which 0·0005 gramme constituted a minimal lethal dose, dissolved in 1·31 c.c. of distilled water, in the jugular vein at 11-35 A.M. Respiratory symptoms, in the shape of gaping and gasping inspirations, set in at once, and it presently fell down. Two minutes after the injection general convulsions set in, and a minute later death occurred. Blood from the throat was immediately received into a sterilised glass capsule. It was of intensely dark, almost black colour, and for a long time remained perfectly fluid. At 12-15 P.M. the surface had hardly undergone any perceptible brightening in colour, and, although coagulation had now occurred, the clot was semi-fluid, and no separation of serum had occurred. An hour later it was in the same state, and on the following morning, it was perfectly black throughout and formed a very soft loose coagulum from which no serum whatever had separated.

The results of this series of experiments appears clearly to show that cobra-venom does produce certain definite, specific effects on any mass of blood with which it may come into contact, either without or within the body. When the proportion of venom to blood is relatively very high, it gives rise to an immediate and total loss of coagulability and of respiratory property accompanied by destruction of all save the nuclear portions of the red corpuscles; where it is lower, it gives rise to similar results only neither so completely nor so rapidly. The action, however, remains alike in nature whatever the ratio of venom to blood may be, and whether it occurs without or within the body, and the amount of venom which is ultimately capable of affecting very considerable changes in relatively very large masses of

blood is fractionally minute. It is evident from the results of experiment VIII that a unit of dried venom is capable of affecting a mass of blood weighing at least 52,000 times as much to such an extent as to practically abolish its respiratory property entirely, which, as we have already seen, is by no means necessary in or der to induce death by asphyxia. A loss of 30 per cent. of respiratory property is sufficient to do this, and, consequently, the experimental data go to show that a unit of dried venom is capable of affecting at least 156,000 times its weight of blood to such an extent as to interfere with its respiratory property to a fatal extent. But the average amount of dried material to be obtained from any individual cobra as the result of a single ejection of venom is 0·254 gramme, an amount sufficient according to these data to produce fatal change in about 40 kilos of blood, and, consequently, on the ordinarily accepted estimate that the blood of an animal contributes one thirteenth part of total body weight, sufficient to cause the death of an animal weighing 520 kilos, or about seven times the weight of any average human subject. This estimate, moreover, may understate the potency of the venom very considerably, as the experimental data do not necessarily indicate its maximal ferment-power.

The data go to show that cobra-venom, not only affects the respiratory property of the blood, but likewise its coagulability, and at the same time acts on the red blood corpuscles in solvent fashion. The observations which have been frequently and quite correctly recorded regarding the absence of any appreciable change in the microscopical features of the blood of animals which have died under the influence of cobra-venom, may appear to be in direct conflict with these data, implying that it possesses a solvent property on the bodies of the red corpuscles; but it is very doubtful whether they really are so. There can be no question that the presence of a solvent property appears very conspicuously where a relatively large amount of venom acts on a limited bulk of blood, or where smaller quantities are given full time to produce their ferment effects upon relatively large bulks; but it does not necessarily follow that they should manifest themselves equally clearly in the blood of animals which have died in consequence of the introduction of the venom into the system in ordinary quantities and in the normal fashion, more especially in cases where the red corpuscles are non-nucleated and where, consequently, free nuclei are not present as an index to the occurrence of processes of solution. In the first place, death must necessarily take place long before the blood has undergone such profound alteration as that which is present when a limited amount of venom has been allowed time to produce its full effects on it; and, secondly, an amount of venom quite capable of producing fatal changes in the respiratory properties of the blood may be insufficient to occasion much solution of the red corpuscles, even if it be allowed time to exert its ferment action to the utmost. The presence of a solvent action may readily escape notice, because the examination of the blood has been conducted ere sufficient time has elapsed to render its effects conspicuous,

or the evidence of any considerable solvent effect may be permanently absent, because, in order to effect the occurrence of any readily appreciable solution, it may be necessary that an amount of venom much in excess of that sufficient to produce death should be present.

The data regarding the effects produced on coagulation show that when a relatively very large proportion of venom comes into contact with a mass of blood, coagulability is at once entirely abolished, and that smaller proportions, although incapable of entirely preventing the occurrence of coagulation, delay its onset, and affect the normal processes of contraction of the clot in degrees varying in direct ratio with their amount. Here there is no even apparent conflict with the phenomena recorded as present in cases of death from cobra-venom. In experiment XI, in which a relatively large quantity of dried venom was directly introduced into the circulation, coagulation was excessively delayed and extremely imperfect, and according to Richards the blood of the lower animals normally remains permanently fluid "when a large quantity of cobra-poison has been directly injected into the circulation, as, for example, into an artery or vein."* But from the experiments it is also quite clear that the respiratory properties of the blood may be profoundly affected by amounts of venom which are quite insufficient to prevent the occurrence of coagulation, and thus that there are no good grounds for expecting that the blood after death from cobra-bite should invariably be incoagulable. On the other hand, it is on record, first, that the blood of animals generally is incoagulable where death has followed the direct introduction of relatively large quantities of venom into the vascular system; and, second, that that of the human subject is normally incoagulable after death from cobra-bite. The first of these two phenomena is clearly in complete accord with the results obtained by causing cobra-venom to act on the blood outside the body, and which demonstrate that large proportions of venom immediately abolish both respiratory property and coagulability, whilst smaller proportions give rise to excessive loss of respiratory property without apparently proportionately affecting coagulability. From the data we should naturally be led to assume that coagulability should, as a rule, be present after death from cobra-bite, but absent where excessive quantities of the venom have come into play. The fact that in cases of death from cobra-poison in the human subject the blood should, as a rule, be incoagulable appears at first sight to be anomalous as there are no good grounds for believing that a bite normally introduces a larger amount of venom into the human body than it does into that of any of the lower animals. There appears, however, to be a possible explanation of it; for, in very many instances at all events, life is more or less artificially prolonged in the human subject after the injection of the venom into the system, by means of various methods of treatment calculated to delay the onset of the comatose

* Report of the Indian Commission for the investigation of Snake-poisons, page 45.

stage of intoxication, and hence there is not merely a chance of a larger absorption of venom into the vascular system before the occurrence of death, but more time is given for that which has been absorbed to produce its effects on the blood. It is very unfortunate that there should be no satisfactory record of the condition of the blood in the case of lower animals in which life has been prolonged by means of artificial respiration, but in the only case in which I can find any note regarding the subject it is stated that partial incoagulability was present.*

There are thus no satisfactory *a priori* grounds for denying that cobra-venom acts by producing changes in the blood, and there is positive experimental evidence that it has the power of causing important changes, and specially respiratory changes in it, and that, owing, apparently, to the action being of a ferment character, such minute amounts of venom are capable of profoundly affecting such relatively large masses of blood that there is no difficulty in accounting for the death of even very large animals from cobra-bite as essentially dependent on blood-change. On the other hand we have seen that the evidence on which the belief in the primary neurotic action of the poison is founded is not of a conclusive character, as the phenomena which are regarded as indicative of such action are not in any way in conflict with the theory that the primary action is a hæmic one. Experiment unequivocally demonstrates the presence of a powerful hæmic action, and the nervous phenomena which present themselves in the course of the intoxication are of a nature parallel to that of those which we find arising in cases of poisoning in which there can be no question that a loss in respiratory property of the blood is the primary factor in the production of disease, why, then, should it be affirmed that the venom acts essentially as a primary and direct nerve-poison?

Wall goes carefully into this question, and it may be well to consider the value of the arguments which he adduces in order to justify his conclusion that, "though cobra-venom is a nerve-poison of surpassing deadliness, as a blood poison it is not an agent of much power."† His arguments are the following:—

I.—Death from anything save nervous symptoms is unknown.

II.—In cases of recovery the subject at once regains normal health after the nervous symptoms have passed off.

III.—In most cases there is no symptom of serious blood-poisoning even whilst the nervous symptoms are present.

IV.—The kidneys give no evidence of altered relation to the blood.

V.—The microscope affords no evidence of structural changes in the blood of animals which have died from the effects of the venom.

With regard to the first of these arguments it is sufficient to point out that,

* Report of the Indian Commission, Appendix No. I, p. VI.

† Indian Snake Poisons, Their Nature and Effects, by A. J. Wall, M. D., 1883, page 47.

if it is to be regarded as an evidence of the absence of blood-change in cases of cobra-poisoning, it must hold the same place in regard to cases of CO-poisoning in which, as a matter of fact, we know that important blood-change is present. The second one has no weight whatever in the absence of proof that the nervous symptoms are dependent on a direct action of the poison on the nervous apparatus. It is only an argument if it be assumed that such an action is present, for, if the nervous symptoms be dependent, as they probably, in greater part at all events, are on defective respiratory property in the blood, their disappearance implies the disappearance of the abnormal hæmic condition causing them. If the damage to respiratory property caused by a dose of venom falls short of causing death, the blood must naturally gradually regain its normal properties, possibly in part as the result of the recovery of respiratory property by red corpuscles which have only, partially suffered, and, in any case, certainly owing to the constant addition of new red corpuscles, and when it has regained its normal respiratory properties the symptoms must naturally disappear. Exactly the same thing occurs in many cases of intoxication by CO in which the blood has not been so far affected as to cause death. After removal from the atmosphere containing the gas, nervous symptoms continue to be present for some time, but they gradually wear off and then no symptoms of blood-change present themselves because no blood-change is present. The results of the following experiment very clearly illustrate this.

Experiment XII.—A fowl was introduced into an aspirating chamber into which a stream of CO was passed for some time. It very soon became profoundly affected, making conspicuous swallowing movements, gaping and inspiring laboriously. It shortly became unable to stand and sat down with its head drooping more and more forward. It was removed from the chamber in this state and gradually recovered so far as to be able to stand up in a tottering fashion and to raise its head. It still, however, was very drowsy and continued to make conspicuous swallowing movements.

On the following day recovery had very greatly progressed and it was feeding freely. Conspicuous swallowing movements were still, however, frequent, and occasionally it appeared as though it were imitating the initial stages of intoxication by cobra-venom, as it became very drowsy, the eyes closing, and the head gradually drooping forward until the lip of the beak rested on the floor and then being suddenly and abruptly elevated.

On the following day recovery was almost complete and soon all abnormal symptoms disappeared entirely.

In this case it is clear that nervous symptoms continued to be present so long as the blood was defective in respiratory property and disappeared when it had regained its normal condition, the phenomena being precisely parallel with those presenting themselves in cases of intoxication by subminimal lethal doses of cobra-venom.

Wall's third argument is of much the same quality as the second one, for until it has been definitely shown that the nervous symptoms are not induced by blood-change, the absence of other evidence of such change is of no conclusive value. As regards the next point, the absence of renal evidence of blood-change, we find that in cases of CO-poisoning there is no constant renal evidence of the blood-change which is unequivocally present, and, on the other hand, that in many cases of poisoning by cobra-venom there is clear evidence of either abnormal increase or decrease of renal secretion. Finally, in respect to the microscopical evidence, it has already been pointed out that the blood derived from the bodies of animals which have just died of intoxication by cobra-venom may very likely show no appreciable signs of alteration in its formed constituents, because death occurs at a time when the venom has not yet exerted its full action, and may be caused by an amount of venom, which, though incapable of producing conspicuous solvent effects on the corpuscles, is amply sufficient to induce a fatal depression of the respiratory property of the blood.

None of the above arguments, then, appear to be of any really conclusive value in demonstrating the absence of any important hæmic change in cases of intoxication by cobra-venom, and I am not aware of the existence of any of greater cogency. On the other hand, there are a whole series of phenomena which appear to be unequivocally adverse to the belief in the primarily neurotic action of the poison.

Wall himself, in spite of his belief, adduces a very important one when he points out that a latent period normally intervenes between the entrance of a lethal dose of venom into the circulation and the appearance of any nervous symptoms, and quite correctly affirms that this shows "that the mere presence of the poison in the blood, even in sufficient quantity to kill, is not capable of producing directly a physiological effect."* But, if it be incapable of producing a direct "physiological effect" on the nervous apparatus, what becomes of its action as a direct nerve-poison? When a lethal dose of any direct nerve-poison enters the circulation it manifests its presence at once and produces its fatal effects with great rapidity. Minimal lethal doses of the salts of strychnia or of one of the toxins which are present in Daboia-venom on entering the circulation cause almost immediate death, but this is not the case with minimal lethal doses of cobra-venom, for their entrance is followed by a prolonged latent period during which no appreciable symptoms whatever are present, and those which subsequently appear follow a very slow and gradually cumulative course ere they terminate in death. The contrast between the phenomena presenting themselves in such cases is illustrated by the results of the following experiments in all of which the poisons were directly

* Op. cit., p. 43.

injected into the jugular vein, and were, therefore, placed in like circumstances as regards the production of their specific effects.

Experiment XIII.—Direct introduction of 0·0044 gramme of nitrate of strychnia into the jugular vein of a fowl.

A fowl received an injection of 0·0044 gramme of nitrate of strychnia in 0·5 c.c. of distilled water directly into the jugular vein. Immediate general convulsions occurred and were followed by death within a few minutes.

Experiment XIV.—Direct introduction of a minimal lethal dose of Daboia-venom into the jugular vein of a fowl.

A sample of dried Daboia-venom was carefully tested and the minimal lethal dose necessary to give rise to fatal general convulsions was ascertained to amount to between 0·004 and 0·005 gramme. A fowl received an injection of 0·005 gramme in 0·5 c.c. of distilled water directly into the jugular vein.

Furious general convulsions set in before the bird could be set down and were immediately succeeded by death.

Experiment XV.—Direct introduction of a minimal lethal dose of dried cobra-venom into the jugular vein of a fowl.

A fowl received an injection of 0·0005 gramme of a stock of dried venom, of which that amount constituted a minimal lethal dose for fowls of 1 kilo in weight. The venom was dissolved in 0·5 c.c. of distilled water and the solution directly injected into the jugular vein at 10-50 A.M. No specific symptoms whatever manifested themselves until between 2 and 3 P.M., and death did not occur until between 7 and 8 P.M.

The entire absence of any appreciable latent period in the case of the entrance of minimal lethal doses of direct nerve-poisons into the circulation, and the necessary intervention of such a period where nervous symptoms are dependent on certain preliminary effects of the poison on the system could hardly be more strikingly illustrated than they are by the results of these experiments. The fact that when relatively excessive quantities of cobra-venom are directly introduced into the circulation the latent period is very greatly abbreviated or even practically entirely absent is, of course, no valid argument against the belief that the primary action is a hæmic one, it only indicates that the toxin is of the nature of a ferment, which, when acting on the blood in large quantities, is capable of rapidly inducing changes which can only be slowly and gradually induced by fractional ones. The phenomena in the two cases are precisely parallel to those which present themselves when large and fractional quantities of venom act upon blood outside the body. Where the proportion of venom is relatively excessive an immediate and total loss of respiratory property and coagulability, and exceedingly rapid solution of the bodies of the red corpuscles take place, where it is fractional, equally great changes ultimately occur in so far, at all events, as respiratory property and solution of the corpuscles are concerned, but their progress is protracted and cumulative.

The phenomena characteristic of the action of minimal lethal doses of cobra-venom are not those of the action of any material which when present within the system at once manifests its presence by producing its maximal effects, but are those of one acting in the manner of a ferment and producing a gradually cumulative effect. The extreme disproportion between cause and ultimate effect in cases of fatal intoxication by minimal lethal doses of the venom, and the fact that fractional doses are capable of ultimately producing the same results as very much larger doses produce within a very short time, also point to the presence of a ferment action, which it is hard to conceive of as coming into direct play upon the nervous apparatus, but which may well ultimately affect the latter by primarily inducing changes in the blood.

Again, it is apparently universally admitted that the immediate cause of death in cases of intoxication by cobra-venom is asphyxia. Those who regard the venom as a direct neurotic poison ascribe this solely to gradual paralytic cessation of respiratory movements, but there are at least two good grounds for doubting the correctness of this conclusion. These are supplied by the phenomena following the direct introduction of relatively large masses of venom into the circulation, and those attending treatment by means of artificial respiration. The result of the introduction of relatively large quantities, of venom directly into the circulation is not as is very clearly shown by the data of Experiment XI, the immediate induction of paralysis, but the immediate induction of respiratory symptoms and of asphyxial convulsions accompanied by profound changes in the blood as evinced by permanent abolition of respiratory property and defective and retarded coagulation. The occurrence of asphyxial convulsions is a normal terminal phenomenon in all cases of acute fatal intoxication by cobra-venom, and they are ordinarily credited solely to the gradual, antecedent failure in respiratory movements, but in cases, like that recorded by Wall in which "violent and irregular contractions of the respiratory muscles" suddenly replaced normal respiration thirty seconds after the injection of 1 c.c. of fresh venom into the saphena vein of a dog,* there is no such antecedent failure to account for the phenomenon and it can only be interpreted as indicating the effects of sudden and excessive diminution in the respiratory property of the blood upon the respiratory centre. The results of the direct introduction of relatively large quantities of venom into the circulation are precisely those which might logically be expected to occur on the theory that the material acts as a blood-poison, but are quite inexplicable by that which regards it as a direct nerve-poison inductive of paralysis.

The phenomena which are present in cases in which treatment by means of artificial respiration is employed are no less suggestive. There appears to be no question that the venom produces no appreciable special effect on the circula-

* Op. cit., p. 36.

tion. Wall states that "cobra-poison cannot be said to produce a very great effect on the circulation. The heart can be kept acting, and the blood circulating for very many hours after the rest of the functions of the body have been suspended, if only artificial respiration is continued. In ordinary cases of cobra-poisoning the heart can generally be felt acting for a short time after respiration has ceased."* But if this be so and death be due to asphyxia purely dependent on diminution and cessation of respiratory movements, how does it happen that death invariably ultimately occurs in spite of the continuous application of artificial respiration? The entire failure of artificial respiration to do more than delay the occurrence of asphyxia is surely sufficient to suggest that the latter cannot be purely dependent on failure of respiratory movements, and, when taken along with the demonstrable effects of the venom on the respiratory property of the blood, leaves little room to doubt that the fatal asphyxia which normally occurs in cases in which artificial respiration is not applied is only partially dependent on defective exposure of the blood to atmospheric oxygen. Under normal circumstances such defective exposure does come into play to hasten the fatal event, but, even where it is not present, as in cases where artificial respiration is applied, asphyxia occurs although after a longer interval. The phenomena appear clearly to indicate that in the one case two factors productive of asphyxia, defective respiratory property in the blood and defective exposure of the latter to the air, are present, whilst, in the other, the former alone comes into play. Artificial respiration apparently acts by giving the residual respiratory property of the blood an opportunity of securing the addition of fresh supplies of oxygen from the air, but it in no way affects the progressive ferment-action of the venom. The latter goes on steadily diminishing the respiratory property of the blood until it falls below the limit which it must attain in order to supply the respiratory demands of the system, and then death naturally occurs in spite of the continued exposure of the fluid to atmospheric oxygen.

Another argument in favour of the belief in the presence of blood-change as the primary result of the action of the venom and the essential cause of the characteristic symptoms is afforded by the results attending treatment by repeated injections of solutions of salts of strychnia. Were the primary action of the venom, as is ordinarily supposed, one directly affecting the nervous centres and giving rise to depression of their functional activity and consequently to paralysis, it would be somewhat hard to explain why it is that treatment with strychnia should have no appreciable effect whatever on the course of the intoxication. But there can be absolutely no question that it has none, and, more than this, that whilst this is the case—whilst the introduction into the system of relatively large quantities of strychnia has no effect whatever on the action of cobra-venom—the effects produced by the latter are to some

* Op. cit., p. 38.

extent repressive of the normal manifestation of the symptoms connected with the action of strychnia on the nervous apparatus, as is clearly indicated by the results of the following experiment.

Experiment XVI.—A fowl received an injection of 0·001 gramme of dried venom, of which 0·0005 gramme constituted a minimal lethal dose for fowls of 1 kilo in weight.

The injection was administered at 10-55 A.M. and at 1-10 P.M. the characteristic symptoms were already highly developed. An injection of 0·0044 gramme of nitrate of strychnia was now administered. The sumptoms of the action of the venom continued to progress and a few slight jerking movements of the tail indicated the action of the strychnia. The symptoms of cobra-poisoning continued to run their ordinary course and death occurred at 4 P.M.

At 1-11 P.M. another fowl received an equal dose of strychnia without any previous treatment. At 1-14 P.M. jerking movements set in followed by violent general convulsions at 1-15 P.M. and death at 1-20 P.M.

Here an amount of strychnia sufficing under ordinary circumstances to give rise to violent general convulsions and death in the course of a few minutes was quite incapable of doing so in the presence of intoxication by cobra-venom. It merely served to produce barely appreciable and temporary specific effects and otherwise in no way affected the normal progress of the intoxication. The phenomena certainly indicated abnormal depression of functional activity in the nervous centres in connection with the intoxication, but afforded no evidence whatever that this was directly dependent on the local action of the venom. On the contrary they were precisely of the character which they might naturally have been expected to present on the theory that the depression of nervous functional activity is dependent on some general cause such as defective respiratory property in the blood. Such a condition, as we know from the phenomena of intoxication by CO does give rise to such depression, and with the presence of the latter it is easy to see why abnormally large quantities of any direct nervous stimulant such as strychnia should have to be introduced into the system in order to occasion specific effects. But there are no logical grounds for supposing that the introduction of any direct nerve-poison should in any way necessarily affect the primary specific action of any ferment-material leading to progressive changes in the quality of the blood. These changes are an effective agent in giving rise to depression of nervous functional activity, but no direct stimulation of the latter by means of excessive doses of strychnia can be expected to touch the primary source of mischief. Death may be hastened by the employment of very large doses of strychnia, but no amount of strychnia will prevent the intoxication due to the venom from steadily progressing towards its normal termination, because no amount of strychnia is capable of arresting the cumulative ferment-action taking

place in the blood. The effects of the action may be temporarily and partially masked by those induced by strychnia, but there is no evidence whatever of the existence of any true physiological antagonism between the two poisons, and, if this be so, the phenomena certainly afford no support whatever to the view that the venom acts as a direct nervous depressant, whilst they are readily accounted for by that which regards it as a primary blood-poison.

The older advocates of the theory that the venom acts as a primary blood-poison laid much weight on the phenomena of variations in susceptibility which are presented by different kinds of animals, and to a certain extent they were right in doing so. Their data were certainly not always absolutely correct, but the same holds good for those which have been advanced by their opponents. For example, Boag affirms that "the poison of the serpent has most power over those animals whose blood is the warmest and the action of whose heart is the most lively; while, on the contrary, it is not a poison to the snake itself, nor in general to cold-blooded animals," and Richards, in commenting on this statement, says: "Mr. Boag observes that a poisonous snake is protected from the effects of its own poison by its physical conformation, which enables the animal to live with a very small amount of oxygen. Unfortunately for this argument, however, venomous and non-venomous snakes do not differ anatomically, and yet the venom of the former will kill the latter." * Now Boag was no doubt in error in supposing that all snakes are absolutely exempt from the action of venom, but Richards is equally wrong in implying that non-venomous snakes as a rule, are as susceptible to the action of venom as animals with more active circulation and respiration and consequently demanding a greater supply of oxygen. Had he shown that non-venomous snakes are normally as susceptible to the action of venom as animals possessing more active respiration and circulation are, his position would have been a strong one, but the mere fact that they are liable to be killed by venom is no argument whatever against Boag's theory, whilst any evidence showing that they are characterised by minor susceptibility is a perfectly valid one in its support. The theory assumes that the essential action of the poison is to diminish the respiratory property of the blood, but it certainly does not imply that such a change ought not to occur in the case of non-venomous snakes. Boag's error lay in generalising on the phenomena presented in the case of venomous snakes in which we have to deal with an immunity which is certainly not dependent solely on minor respiratory requirements, but also on the presence of a factor interfering with the ferment-action of the poison. In the case of non-venomous snakes and other harmless reptiles with low respiratory requirements the theory does not demand the presence of any absolute immunity but only of a minor degree of susceptibility. It is clear that

* Report of the Indian Commission on snake-venom, page 8.

a reduction in the respiratory properties of the blood sufficing to cause death in animals of high respiratory requirements will not be sufficient to kill others of much lower respiratory requirements, but it does not necessarily follow that the latter should possess any absolute immunity, for, if the amount of venom be increased in proportion to the defect in respiratory requirement, there can be no reason why a fatal effect should not be produced. All then that the theory requires is that non-venomous snakes and similar animals should be relatively insusceptible, and there is good ground for believing that they are so and that degree of susceptibility, so far, runs parallel with that of respiratory requirement.

In the first place it can be readily shown that certain unequivocally harmless reptiles possess an extraordinarily high power of resistance to the action of cobra-venom. Those in regard to which this has been most conclusively demonstrated are *Zamenis mucosus*, the common "Rat-snake" or Dhamin of India, and *Varanus salvator*, the large water-lizard, which abounds in moist regions in the peninsula. Sir Joseph Fayrer was the first to point out that *Zamenis mucosus* possesses at all events a very high relative immunity, and was perfectly correct in doing so in spite of the imperfection of his data in regard to the actual quantity and quality of the venom which failed to produce a fatal result and the weight of the snakes to which it was administered, and of the fact that, as a rule, the snakes were not kept long enough under observation to show conclusively that the exemption was a permanent one. The following experiments, the results of which are entirely confirmatory of his conclusions, were, however, free from such sources of error.

Experiment XVII.—A large Zamenis, weighing 888 grammes, received an injection of 0·01 gramme of dried cobra-venom, of which 0·0005 gramme constituted a minimal lethal dose for fowls of 1 kilo in weight. No symptoms of any kind followed the operation either at the time or during a succeeding period of several months' duration during which the snake was kept under observation.

Experiment XVIII.—A Zamenis, weighing 903 grammes, received an injection of 0·05 gramme of the dried venom employed in the previous experiment. No symptoms whatever showed themselves during the course of several succeeding months.

Experiment XIX.—A Zamenis, weighing 663 grammes, received an injection of 0·05 gramme of the same dried venom as that employed in the previous experiments with the same result as was present in them.

Experiment XX.—A Zamenis, weighing 788 grammes, received an injection of 0·05 gramme of the same venom with like results.

Experiment XXI.—A Zamenis, weighing 720 grammes, received an injection of 0·1 gramme of the same dried venom with like results.

In the first of the above experiments the snake received an amount of venom sufficient to kill 20 fowls, in the next three an amount sufficient to kill 100, and in the fifth enough to kill 200 fowls of 1 kilo in weight without showing any morbid symptoms either at the time or for several months subsequently. There can therefore be no doubt whatever of the presence of very high relative insusceptibility in this species of snake of the absolute harmlessness of which there can be no doubt. The next question is, how far does this coincide with low respiratory requirement? The following experimental data satisfactorily answer this by showing the results attending the exposure of fowls and specimens of Zamenis to like conditions calculated to affect the respiratory properties of the blood.

Experiment XXII.—Effects of submersion in water.

A fowl and a large Zamenis were enclosed in separate cages and submerged in a large vessel of water. The fowl was taken out dead after the lapse of three minutes, but the snake remained apparently unaffected for a much longer time, did not cease struggling until 23 minutes and a half after submersion, and, when removed from the water, gradually revived and was presently none the worse for the treatment.

Experiment XXIII.—Effects of exposure to an atmosphere containing a large proportion of CO:—

(*a*) A fowl was enclosed in a chamber through which a current of CO was drawn by means of an aspirator. Shortly after the beginning of the experiment the bird began to make frequent and conspicuous swallowing movements. These were soon followed by gaping of the mandibles and staggering gait. Soon after it sat down, with the beak permanently gaping, and laboured deep respiration, the head intermittently dropping forward and being suddenly elevated again. The mandibular gaping soon assumed an intermittent type and the head gradually became permanently depressed and the neck so much flexed that the vertex rested on the floor of the chamber just as it does in cases of intoxication by cobra-venom. Slight general convulsions set in 48 minutes after the commencement of the experiment and death followed almost immediately.

(*b*) A large Zamenis was introduced into the same chamber and treated in the same fashion as the fowl. It never showed any signs whatever of discomfort, and when removed from the chamber after the lapse of an hour was quite lively and seemingly unaffected in any way.

(*c*) A large Zamenis was put into the aspirating chamber and treated as above. It remained in the chamber for two hours without showing any symptoms of being in any way affected and when removed from it was quite lively.

The evidence of the coincidence of minor respiratory requirement with relative insusceptibility to the action of cobra-venom is thus quite unequivocal in this instance, and that which is afforded by the experimental data regarding *Varanus salvator*, which are given below, is quite as clear.

Experiment XXIV.—A Varanus, weighing 2,600 grammes, received an injection of 0·0125 gramme of dried cobra-venom, of which 0·0005 gramme was a minimal lethal dose for fowls of one kilo in weight. It never showed any symptoms whatever of being affected by the poison.

Experiment XXV.—A Varanus, weighing 2,483 grammes, received an injection of 0·03 gramme of the same dried venom and never showed any appreciable symptoms of its action.

Experiment XXVI.—A Varanus, weighing 2,360 grammes, received an injection of 0·1 gramme of dried cobra-venom of the same lethal property as that employed in the previous experiment and died about seven hours later.

Experiment XXVII.—Effects of submersion in water. A Varanus of average size was submerged in water and died three hours and forty minutes later.

It is quite clear then that in some cases a high degree of insusceptibility to the action of cobra-venom is present in association with a low degree of respiratory requirement, but, at the same time, that even in the case of certain non-venomous reptiles the latter cannot be the sole cause of the exemption, for Dhamins, which, are much more rapidly drowned than Varani, possess a far higher exemption than the latter do. In the above experiments on submersion twenty-three and a half minutes was sufficient to reduce a Dhamin to the point of death, whilst it took more than nine times as long to drown a Varanus, and yet a dose of 0·1 gramme of dried cobra-venom was sufficient to kill a Varanus weighing 2,360 grammes, and one of equal amount was incapable of producing any appreciable effect on a Dhamin of 720 grammes weight. The dose which the Varanus received was equivalent to one of about 0·05 for each kilo of body-weight, an amount, which, with venom of the lethal property of the stock from which it was derived, would certainly have killed any fowl of approximately 1 kilo in weight within an hour, whilst here it took about 7 hours to cause death. This and the results of the other experiments on *Varanus salvator*, show that the species is endowed with a relative insusceptibility as compared with fowls which may fairly be credited to the presence of much lower respiratory requirement, but this is no longer the case with that of Zamenis which with much higher respiratory requirement than Varanus has an incalculably greater exemption.

It remains to be considered how far there is any evidence to show that the coincidence of low respiratory requirement with relative insusceptibility is not an exceptional one, but in regard to this point the data are unfortunately very defective. A large number of experiments have, no doubt, been tried in regard to the action of cobra-venom on non-venomous snakes, but they all labour under

the defect of yielding no accurate data in regard to relative susceptibility. They have all been devoted to determining whether it is possible to kill harmless snakes with venom—to ascertaining the presence or absence of absolute not of relative immunity—and, on this account, the data which have been recorded regarding them afford no accurate information as to the precise relation of amount of venom and body-weight of the recipient animal, and without information on this point it is of course impossible to determine the degree of relative immunity or susceptibility with any accuracy.

In the only experiment on harmless snakes other than Dhamins which I have myself conducted, the subject was a *Tropidonotus piscator* of moderate size. It received a dose of 0·05 gramme of dried venom, of which 0·0005 gramme constituted a certain minimal lethal dose for fowls of about 1 kilo in weight. No abnormal symptoms of any kind presented themselves during the day on which the venom was administered or on the two following ones. The snake continued to be perfectly lively and seemingly quite unaffected until the night of the third day, but was found dead on the following morning. Taking the entire absence of any gradual development of symptoms, along with the fact that the species is a very delicate one in confinement, it appears to be very questionable whether the fatal result were in any way dependent on the specific action of the venom; but, even if it be assumed that it was, the phenomena indicate the presence of relative immunity of high degree, seeing that the snake certainly could not have weighed above half a kilo and that an amount of venom sufficient to kill a fowl of one kilo in weight in thirty-four minutes or so produced no appreciable symptoms whatever for at least sixty hours.

Waddell in a paper which appeared in Part IV of the present periodical* tabulates the results of a series of experiments which he carried out in regard to the question of the presence or absence of absolute immunity in various species of harmless snakes, and although his data do not allow of any definite conclusions regarding the precise degree of susceptibility present in individual instances, they appear quite clearly to indicate the presence of relative insusceptibility. The table includes the results of sixteen experiments. The weights of the snakes which were employed are unfortunately not given but their lengths are, and an approximate estimate of their weights has been derived from the latter by taking the average weight of Dhamins of at least five feet in length and using this as a standard in attempting to calculate the ratio of weight to length in the other species. The results attainable in such a fashion are, of course, merely approximate ones, but the error probably lies in an over, rather than an under estimate of weight to length, as the Dhamin is by no means a specially slender snake, and some of the specimens from which the average weight was obtained were considerably over

* Are venomous snakes auto-toxic. Surgeon L. A. Waddell.

five feet in length, whilst the length taken as the standard in the calculation was only five feet. Working on this principle it appears that the total weight of the snakes in Waddell's experiments must have been approximately 5,188 grammes, and the data regarding the weights of venom show an expenditure of about 1·049 gramme. The average weight of the snakes according to this must have been 324 grammes and the average dose of venom 0·065 gramme. But the average duration of life after the administration of the venom was apparently about six hours, and this clearly shows high relative resistance to the action of the poison seeing that a dose of 0·05 gramme of dried venom of good average quality is sufficient to kill a fowl of one kilo in about half an hour. In other words, an amount of venom more than sufficient to kill fowls of one kilo within half an hour only sufficed to kill snakes of less than half that weight in about six hours, or the average dose administered to the snakes taken in relation to their weight was equivalent to one of at least 0·2 gramme to a fowl of one kilo in weight, and yet death did not follow for about six hours. These results are only to be taken as roughly approximate ones, but, such as they are, they afford very conclusive evidence of the existence of relative insusceptibility in snakes generally.

Whilst snakes as a group appear to be relatively insusceptible to the action of cobra-venom, this is certainly not the case with Lacertilia in spite of the fact that certain species, such as *Varanus salvator*, enjoy a relative immunity. This comes out very clearly from the results of the following experiments on the effects following the administration of venom to *Calotes versicolor*.

Experiment XXVIII.—A Calotes versicolor, weighing 30 grammes, received an injection of 0·00025 gramme of dried venom of which 0·0005 gramme constituted a minimal lethal close for fowls of about one kilo in weight.

Death occurred 21 minutes after the introduction of the venom. The dose of venom was equivalent to one of 0·00825 gramme to a fowl of one kilo in weight, an amount, which, with venom of the lethal property which this possessed, would induce death in less than half an hour.

Experiment XXIX, a Calotes versicolor—Weighing 22 grammes, received an injection of 0·000062 gramme of dried venom and died about two and a half hours later. Here the dose was equivalent to one of 0·00279 gramme to a fowl of one kilo in weight and the period of survival corresponded approximately to that ordinarily present after the administration of such an amount.

According to these data *Calotes versicolor* is practically as susceptible to the action of cobra-venom as fowls are. Neither relative immunity nor high susceptibility, as has been affirmed by certain observers,* is, therefore, a general Lacertilian peculiarity. Relative immunity is present in very high degree in

* "Étude expérimentale du Venin de Naja tripudians" par Le Dr. A. Calmette. Annales de L'Institut Pasteur, No. 3, page 160.

Varanus, whilst great susceptibility replaces it in Calotes. But there are other very conspicuous coincident differences present in the two cases, for Calotes is a purely terrestrial and very active animal, whilst Varanus is essentially aquatic and sluggish, and whilst a submersion of nine minutes' duration serves to render a *Calotes* motionless and insensible, one of more than three hours is required to produce a similar effect on a *Varanus salvator*. The evidence of the coincidence of susceptibility or relative immunity with high and low respiratory requirement is consequently just as clear in this instance as it is where the phenomena occurring in fowls are compared with those occurring in snakes as a group.

The data obtained from the following series of experiments indicate the presence of relative insusceptibility in Batrachia.

Experiment XXX.—A large specimen of *Rana tigrina*, weighing 200 grammes, received a subcutaneous injection of 0·0005 gramme of dried venom of which that amount constituted a minimal lethal dose for fowls of about 1 kilo in weight. For a long time it appeared to be entirely unaffected, but death occurred on the evening of the fourth day after the introduction of the poison. The dose here was equivalent to one of 0·0025 to a fowl of 1 kilo in weight, but death instead of occurring within an hour or two did not take place for more than four days.

Experiment XXXI.—A small *Rana tigrina*, weighing only 35 grammes, received a subcutaneous injection of 0·0005 gramme of the same dried venom as was employed in the previous experiment and died in forty-six minutes, or in about the same time as fowls which have been treated with doses of venom of only half the equivalent amount die.

Experiment XXXII.—A large *Bufo melanostictus*, weighing 72 grammes, received a subcutaneous injection of 0·0005 gramme of the dried venom employed in the previous experiments and died during the night of the second day after that on which the venom was introduced, whilst a proportionate dose in the case of a fowl would have caused death within an hour.

Experiment XXXIII.—A *Bufo melanostictus*, weighing 60 grammes, received a subcutaneous injection of 0·0005 gramme of the dried venom employed in the previous experiments and died about two hours later.

Experiment XXXIV.—A *Bufo melanostictus*, weighing 29 grammes, received an injection of 0·0005 gramme of the venom employed in the previous experiment and died between four and five hours later.

Experiment XXXV.—A *Bufo melanostictus*, weighing 25·5 grammes, received an injection of 0·0005 grammes of the dried venom employed in the previous experiments, or a dose equivalent to one of 0·019 gramme to a fowl of 1 kilo in weight, and death did not occur until about six hours later.

The above experimental data are all those for which I can personally

vouch, but both Waddell's and Wall's results afford confirmatory evidence of the existence of relative insusceptibility in Batrachia generally. Waddell's table showing the results which he obtained includes eleven experiments on five distinct species.* The amount of dried venom which was employed was about 0·638 gramme which gives an average of 0·058 gramme per dose. But the average weight of fair sized toads and frogs only amounts to about 76 grammes so that the average dose to the average weight of the recipient animals may be fairly taken as equivalent to one of 0·754 gramme to a fowl of, one kilo in weight, and yet the average duration of life subsequent to the treatment was about eight hours even although the venom was directly introduced into the dorsal lymph-sac, and hence practically directly into the circulation, whilst the introduction of equivalent doses directly into the circulation of fowls or other warm-blooded animals would inevitably be followed by almost instantaneous death. Wall's experimental results* in regard to *Rana tigrina* equally clearly indicate the presence of high relative insusceptibility in Batrachia, as in one case death did not occur until five hours after the injection of a solution of 0·01 gramme of dried venom into the dorsal-sac, and in another, in which no less than 0·05 gramme was similarly introduced, it did not take place for more than an hour.

There is little really definite information in regard to the relative susceptibility of invertebrate animals to the action of cobra-venom, but the data which were obtained from a series of experiments on cockroaches afforded little evidence of the presence of any specific effects, for although a large mortality attended the injection of venom, the mortality following the injection of equal quantities of human saliva was also very considerable. The evidence is not of any great value but, such as it is, is rather in favour of the view that the specific action of cobra-venom is one which is exerted upon the blood and which acts by reducing hæmic respiratory property, seeing that in the case of most tracheate animals the blood has no special respiratory function and cannot, consequently, afford the venom a fair field for action. The entire body of evidence relative to the degree of susceptibility to or exemption from the action of cobra-venom in various kinds of animals thus affords very considerable support to the theory that the poison is primarily and essentially a hæmic one, and is at the same time quite inexplicable on the hypothesis of any primary neurotic action.

The principal points of evidence in favour of the theory that cobra-venom acts as a primary blood-poison may be summarised as follows:—

I. Cobra-venom does produce important changes on the blood outside the body.

II. There is clear evidence of the occurrence of similar changes in the

* Op. cit.

blood within the body in cases where large doses of venom are introduced directly into the circulation, or where life is artificially prolonged so as to give time for the ferment-action of smaller doses to be fully exerted.

III. The nervous phenomena which are present in cases of intoxication by cobra-venom are closely parallel to those occurring in cases of intoxication by CO, and the fact that the colour of the blood does undergo alteration on exposure to air is no proof that it has not sustained a lethal alteration in respiratory property.

IV. The fact that, whilst the poison produces no appreciable effect upon the circulation, artificial respiration, although protracting life, is powerless to avert an ultimate fatal result, shows that the asphyxia which causes death cannot be simply dependent on defective exposure of the blood to air.

V. Where minimal lethal doses of cobra-venom are introduced into the system by direct intravenous injection, a prolonged latent period ensues before any specific symptoms manifest themselves, and, when the latter do appear, they follow a gradually progressive, cumulative course, whereas whenever minimal lethal doses of primarily neurotic poisons have entered the circulation they give rise to immediate effects.

VI. Were the phenomena of nervous depression attending intoxication by cobra-venom dependent on a direct action upon the functional activity of the nervous centres, the administration of salts of strychnia ought to produce some appreciable effect on their progress, whereas in fact it produces none whatever.

VII. Were the asphyxial termination of ordinary cases of intoxication by cobra-venom dependent on a gradual, direct depression of the activity of the nervous centres leading to paralytic cessation of respiratory movements, the injection of large quantities of venom directly into the circulation ought to be followed by sudden general paralysis, the quality of the blood remaining unaltered, whereas it is actually followed by sudden general convulsions and loss of coagulability in the blood.

VIII. The theory of the direct action of the venom upon the nervous system is quite incapable of affording any explanation whatever of the conspicuous differences in the degree of susceptibility exhibited by different kinds of animals, whilst that which regards the action as primarily affecting the blood, and specially the respiratory property of the blood, is capable of explaining them so far in a very satisfactory fashion. The neurotic theory might, of course, explain the phenomena of the coincidence of minor susceptibility with minor respiratory requirement, were the former indicated merely by minor mortality, which might be interpreted as owing to the fact that a paralytic decrease in respiratory movement which must necessarily prove fatal in animals with high respiratory requirement, will by no means suffice to do so where the latter is much less. But, as a matter of fact, minor susceptibility is not evinced merely

by minor mortality but by minor tendency to the development of nervous symptoms. The Dhamins in Experiments XVII—XXI remained perfectly lively and vigorous, and showed no nervous symptoms whatever after having been subjected to the action of doses of venom more than sufficient to have caused the full development of all the characteristic symptoms preceding death in from 20 to 200 fowls of corresponding weight, and as we have already seen that the characteristic nervous symptoms are essentially those which attend defective supply of respiratory O, the whole evidence merely goes to show that in the case of the snakes no defect in oxggen-supply sufficient to give rise to any appreciable symptoms was ever present in spite of the presence of excessive doses of a material supposed to act directly on respiratory activity through the nervous system.

II.–The physiological action of the venom of *Vipera russellii.*

All the older observers would seem to have regarded the physiological action of the venom of Russell's viper as essentially identical with that of cobra-venom. Even Fayrer and Brunton, in their communications to the Royal Society, affirmed the practical identity of the symptoms induced by the action of the two venoms, and it was left to Wall to be the first clearly to appreciate that their actions are essentially dissimilar, and that the venom of the Daboia is practically of a more complicated nature than that of the cobra, seeing that it contains, or, at all events, may contain two perfectly distinct toxic materials in lethal amount, each of them possessing independent lethal properties, but neither of them inducing symptoms identical with those attending fatal intoxication by cobra-venom.

It is somewhat hard to understand how any doubt could ever have existed in regard to the different natures of the two venoms, and certainly none could ever have been entertained had their action in minimal or approximately minimal lethal doses been carefully studied. The striking differences in respect both to lethal property and to the nature of the phenomena attending the action of the two venoms which any such study reveals are illustrated by the following data derived from a series of experiments which were conducted simultaneously with samples of venom of ascertained and high lethal property.

A.—Experiments on the action of the venom of Naia tripudians.

As the result of a series of experiments on the properties of two stocks of dried venom, which had been accumulated from many distinct snakes and subsequently thoroughly mixed and reduced to an impalpable powder, it was definitely ascertained that 0·00025 gramme in the one case, and 0·0005 gramme in the other constituted minimal lethal doses for fowls of approximately one kilo in

weight; that doses of 0·000125 gramme in the former, and 0·00025 in the latter, although incapable of causing death gave rise to well marked symptoms; and that smaller doses were followed by no appreciable results.

The results attending the administration of doses of 0·0005, 0·00025, 0·000125 and 0·0001 gramme of the stronger stock of venom were as follows:—

Experiment XXXVI.—10-23 A.M. A fowl received a subcutaneous injection of 0·0005 gramme of dried venom in 0·5 c.c. of distilled water.

10-35. Apparently somewhat drowsy, and sitting down.

10-40. Gaping of the mandibles and gasping, laboured respiration beginning to appear.

The symptoms of intoxication then rapidly ran their ordinary course. Inspiration was at first deep and laboured, accompanied by elevation of the head and conspicuous gaping of the mandibles. The signs of drowsiness continued and increased in degree, and between each inspiratory movement the head gradually tended more and more to droop forward until the tip of the beak rested on the floor of the cage. For a time the eyes were opened and the head suddenly and violently elevated with each inspiration, but presently this ceased to be the case and the bird lay flat with the neck so much flexed that the vertex was in contact with the floor. Futile attempts at raising the head continued for a time to occur at intervals, but they gradually ceased and the bird then lay motionless with the neck extended and the head resting on one side, inspiring deeply at relatively wide intervals. Still later respiratory movements became even less frequent and diminished greatly in extent, and, with this, gradually increasing lividity of the comb and wattles began to appear. Violent general convulsions presently set in and were repeated at frequent intervals until death occurred at 3-45 P.M., or five hours and 22 minutes after the injection of the venom.

Experiment XXXVII.—10-52 A.M. A fowl received a subcutaneous injection of 0·00025 gramme of dried venom in 0·25 c.c. of distilled water.

1-55 P.M. Slight gaping of the mandibles with each inspiration beginning to appear.

From this time onwards the ordinary, specific symptoms of the action of the venom continued slowly and steadily to develope until 1-30 P.M. of the following day when death occurred. No conspicuous convulsive symptoms preceded death.

Experiment XXXVIII.—10-45 A.M. A fowl received a subcutaneous injection of 0·000125 gramme of dried venom in 0·125 c.c. of distilled water.

1-55 P.M. Slight inspiratory gaping beginning to appear.

The normal symptoms attending the action of the venom slowly and gradually developed themselves, and during the forenoon of the following day were well marked, the bird sitting with its eyes closed and the tip of the beak resting on

the floor, save when the head was thrown up with each deep, gaping and gasping inspiration. During the afternoon, however, the symptoms began gradually to diminish in intensity, and from this time onwards recovery steadily progressed, so that, on the morning of the next day, the bird was seemingly almost quite well. The phenomena in this case were almost ludicrously like those succeeding a non-fatal exposure to an atmosphere highly charged with CO.

Experiment XXXIX.—10-50 A.M. A fowl received a subcutaneous injection of 0·0001 gramme of dried venom in 0·1 c.c. of distilled water. No unequivocal symptoms of the specific action of the venom ever manifested themselves.

These and similar experiments clearly demonstrate that the symptoms which are induced by small lethal doses, minimal lethal doses, and sublethal doses, so long as the latter are capable of giving rise to any appreciable results, of cobra-venom are practically alike in character, and that the only phenomena distinguishing the action of larger from smaller doses are that the symptoms appear sooner and are more rapidly developed, and that convulsions immediately before death are more common and conspicuous in cases where the former have been employed. In other words, convulsions immediately before death are more constant and conspicuous in acute than in chronic cases, which, as has already been pointed out, is a phenomenon which is characteristic of intoxication by CO also.

In all cases in which small doses are employed, even in those in which the venom is directly introduced into the circulation, a distinct latent period intervenes ere the development of any appreciable specific symptoms, and the duration of the period clearly holds an inverse ratio to the magnitude of the dose. In experiments, like the above, in which subcutaneous injection is employed it must, of course, remain more or less uncertain how long some of the venom does not remain localised at the site of injection, and at what rate it is removed by absorption into the blood vascular system, and hence in order to obtain definite information in regard to the relative duration of the latent period it is necessary to have recourse to direct introduction of various quantities of venom of ascertained lethal properties into the blood. In the following four experiments this procedure was accordingly adopted, the dried venom which was employed being derived from the stock of which 0·0005 gramme constituted a minimal lethal dose for fowls of approximately 1 kilo in weight.

Experiment XL.—10-50 A.M. A fowl received an injection of 0·0005 gramme of dried venom, in 0·5 c.c. of distilled water, in the left jugular vein.

No symptoms appeared until between 2 and 3 P.M., but they then gradually developed, ran their normal course and terminated in death between 7 and 8 P.M.

Experiment XLI.—11-25 A.M. A fowl received an intrajugular injection of 0·01 gramme of dried venom in 1·5 c.c. of distilled water.

11-35 A.M. Beginning to gape, gasp, and throw up its head with each inspiration.

11-37 A.M. Lying motionless with the neck extended and the head on one side.

11-40 A.M. Shifting about staggeringly at intervals.

11-45 A.M. General convulsions, repeated at intervals.

12-5 Noon. Dead.

Experiment XLII.—10-51 A.M. A fowl received an intrajugular injection of 0·1 gramme of dried venom in 1·5 c.c. of distilled water.

10-53 A.M. Mandibular gaping, and gasping inspiration beginning to appear.

10-56 A.M. The tip of the beak resting on the floor of the cage; the head elevated in characteristic fashion with each inspiration and then dropping forward again; comb and wattles gradually becoming livid.

10-57 A.M. Head falling over on one side.

10-59 A.M. Violent general convulsions; repeated at intervals until 11-1 A.M. when death occurred.

Experiment XLIII.—11-35 A.M. A fowl, weighing 1,550 grammes, received an intrajugular injection of about 0·34 gramme of dried venom in 1·5c.c. of distilled water in the jugular vein.

Respiratory symptoms in the form of mandibular gaping, and laboured, gasping inspiration set in at once.

11-37. Violent general convulsions.

11-38. Dead.

This entire group of experiments very clearly indicates that the essential action of the venom remains unaltered whatever doses of it may be employed, and that practically the only phenomena distinguishing the action of excessive from that of minimal lethal ones is that the former gives rise to maximal effects at once, whilst the latter only does so gradually and progressively. In the case of excessive doses there is no appreciable latent period and death occurs almost instantaneously; in that of doses of somewhat less amount a brief latent period makes its appearance, and the indications of the maximal action of the venom are correspondingly delayed; and in the case of fractional doses the latent period is greatly protracted, and the development of the specific symptoms is very gradual and progressive. So long, however, as a dose remains capable of producing any appreciable symptoms whatever, these are of the same nature as the initial symptoms of excessive doses, and in the case of minimal lethal doses the ultimate state of the victim is precisely alike that almost instantaneously induced by excessive doses, save that convulsive phenomena are not so conspicuous. The differences in the phenomena present in the individual experiments

of this group come out very clearly when they are stated, as below, in tabular form :—

Table showing the course of intoxication following intravenous injections of various amounts of venom.

No. of experiment.	Dose of venom.	Period after administration at which respiratory symtoms appeared.	Period after administration when convulsions occurred.	Period after administration when death occurred.
XL	0·0005 gramme.	Over three hours . .	.....	Over eight hours.
XLI	0·01 „ .	Ten minutes . .	Twenty minutes . .	Forty minutes.
XLII	0·1 „ .	Two minutes . .	Eight minutes . .	Ten minutes.
XLIII	0·34 „ .	At once . . .	Two minutes . .	Three minutes.

The dose of 0·0005 gramme in Experiment XL was just as certainly fatal as that of 0·34 gramme in Experiment XLIII, and the only difference in the two cases was that whilst in the former one a latent period of several hours intervened between the entrance of the venom into the circulation and the manifestation of any specific symptoms of its action, in the latter there was no appreciable latent period whatever and symptoms manifested themselves at once. The initial symptoms were in both cases alike, and alike indicative of defective respiratory supply, but those dependent on the action of the large dose of venom ran a rapid course and terminated in well-marked convulsions and death within a few minutes, whilst those caused by the minimal lethal dose followed a slowly cumulative course in which general convulsions were absent or, at all events, were not conspicuous.

It appears, thus, that cobra-venom is a material the action of which is in no way essentially affected by the amount of it which enters the system. Large quantities give rise to more rapid effects than small ones, and in the case of the latter, a latent period intervenes between the period of access of the venom and the manifestation of any appreciable symptoms of its action, whereas maximal doses give rise to maximal affects at once. The introduction of large quantities of venom into the circulation gives rise to almost instantaneous death, preceded by violent general convulsions; moderate doses are followed by the development of a series of symptoms, which begin to appear after a brief latent period, and culminate in general convulsions and death; fractional doses are incapable of causing any appreciable symptoms until a prolonged latent period has intervened, but the symptoms which then appear are identical in character with those induced by moderate doses, save that general convulsions are neither so constant nor so well marked. But these phenomena surely warrant the conclusion that the essential principle of the venom is of the nature of a ferment and, consequently, not only capable of producing certain effects instantaneously when it enters the system in great excess, but of inducing them gradually and cumulatively when it enters in fractional quantities only.

Two other distinctive properties of cobra-venom are, first, that the power of even minimal lethal doses is in no way appreciably affected by dilution; and second, that the same results ultimately follow the repeated, cumulative introduction of quantities of venom, quite incapable individually of producing any appreciable effects, as those which attend the administration of quantities of lethal amount at one time. That this is the case is illustrated by the results of the following experiments.

Experiment XLIV.—(*a*) 10-25 A.M. A fowl received a subcutaneous injection of 0·001 gramme of dried venom, of which 0·00025 constituted a minimal lethal dose, in 1c.c. of distilled water.

12-5 Noon. Respiratory symptoms beginning to appear.

3-35 P.M. Death occurred.

(*b*) 10-55 A.M. A fowl received a subcutaneous injection of 0·001 gramme of the same dried venom in 5c.c. of distilled water.

12-30 Noon. Respiratory symptoms beginning to appear.

4 P.M. Death occurred.

Experiment XLV.—(*a*) 10-51 A.M. A fowl received a subcutaneous injection of 0·0005 gramme of dried venom, of which that amount constituted a minimal lethal dose, in 0·5c.c. of distilled water.

11-50 A.M. Respiratory symptoms beginning to appear.

8 P.M. Death occurred.

(*b*) 10·30 A.M. A fowl received a subcutaneous injection of 0·0005 gramme of the same dried venom, in 1·5c.c. of distilled water.

1 P.M. Respiratory symptoms beginning to appear.

7 P.M. Death occurred.

Experiment XLVI.— 10·44 A.M. A fowl received a subcutaneous injection of 0·000058 gramme of dried venom, of which 0·00025 constituted a minimal lethal dose, and, subsequently, four other similar injections at intervals of twenty minutes.

2 P.M. Respiratory symptoms beginning to appear.

They slowly and gradually followed their ordinary course, and death occurred at 4 A.M. of the following morning.

The final outcome of the entire series of experiments goes to show that the essential toxic principle of cobra-venom is a powerful ferment-substance, the action of which is neither affected by dilution previous to introduction into the system, or by cumulative introduction in quantities which are individually incapable of giving rise to any appreciable effects, and which is not subject to rapid decomposition or excretion subsequent to absorption. It now remains to consider how far any such conclusions are warranted in regard to Daboia-venom.

B.—Experiments on the action of the venom of Vipera russellii.

Daboia-venom would appear never to be so lethal weight for weight as cobra-

venom, for the normal lethal doses of all the samples of dried venom of the best quality which have yet been tested have ranged from 0·003 to 0·006 gramme, whilst those of potent cobra-venom are only 0·00025 to 0·0005 gramme, or twelve times smaller. But, whilst this is the case, minimal lethal doses of Daboia-venom of the best quality are far more rapidly fatal than minimal lethal doses of the best cobra-venom. The results of the following experiments illustrate the effects following the administration of various amounts of dried venom of potent quality.

Experiment XLVII.—10-45. A.M. A fowl received a subcutaneous injec. tion of 0·001 gramme of dried venom in 1 c.c. of distilled water. Strong muscular twitching occurred at the site of injection. The bird remained during the rest of the day sitting, or standing motionless and apparently somewhat drowsy, but on the following morning showed no appreciable symptoms of any kind, nor did any appear subsequently.

Experiment XLVIII.—10-24 A.M. A fowl received a subcutaneous injection of 0·001 gramme of dried venom in 1 c.c. of distilled water. Well-marked muscular twitching occurred at the site of injection.

10-40 A.M. Sitting down; motionless; very dull

11-40 A.M. Sitting motionless with the head permanently dropped forwards; but no tendency to mandibular gaping, laboured inspiration or closure of the eyes.

The bird remained in this condition until about 4-30 P.M. and then gradually recovered.

Experiment XLIX.—11 A.M. A fowl received a subcutaneous injection of 0·002 gramme of dried venom in 1 c.c. of distilled water. Well-marked muscular twitching occurred at the site of injection. The bird sat down at once and remained perfectly motionless. There were no signs whatever of drowsiness or of respiratory symptoms, but merely extreme, watchful immobility. It remained in this condition until the evening and then gradually recovered.

Experiment L.—10-20 A.M. A fowl received a subcutaneous injection of 0·002 gramme of dried venom in 1 c.c. of distilled water.

There was marked local muscular action at the site of injection. The bird presently sat down and remained perfectly still and very dull almost continuously until 4 P.M. when it began to become livelier, and, by the following morning, it was seemingly quite well.

Experiment LI.—(*a*) 10·32 A.M. A fowl received a subcutaneous injection of 0·003 gramme, of the same sample of dried venom as was employed in the previous experiments, in 1 c.c. of distilled water.

Strong local muscular action occurred at the site of injection. Violent general convulsions set in after a few minutes and lasted until 10-38 A.M. when death occurred.

(*b*) 10-56 A.M. A fowl received a subcutaneous injection of 0·003 gramme of the same dried venom in 3 c.c. of water.

Strong muscular action occurred at the site of injection. The bird sat down motionless and for a time seemed to be very drowsy.

At noon the drowsiness had entirely passed off and it now sat "still as a stone" and watchful, like the birds in the earlier experiments. As in these experiments these symptoms persisted until about 4 P.M. when recovery gradually set in.

Experiment LI.—(*a*) 10-48½ A.M. A fowl received a subcutaneous injection of 0·003 gramme of the same stock of dried venom in 1 c.c. of distilled water.

Strongly marked local muscular action occurred at the site of injection, and for a time the bird seemed to be somewhat excited and restless. It then began occasionally to close its eyes and to make frequent swallowing movements.

10-58. It sat down.

11-1. Violent general convulsions set in and death occurred almost immediately.

(*b*) 11-5 A.M. A fowl received an injection of 0·003 gramme of dried venom in 3 c.c. of distilled water. The solution of venom was the same as that employed in *a* after dilution to the required extent. Well-marked local muscular action attended the injection.

The bird presently sat down and presented the same symptoms as those which occurred in the first four experiments. It did not, however, recover as the birds in the latter did, but continued sitting motionless and died quite quietly during the night.

All the preceding experiments were tried with venom of the most powerful lethal quality; in those which follow the potency of the venom was not so great owing apparently to the fact that at the time the latter was collected the snakes were feeding freely, whereas the more powerful material was procured during the course of the winter and when they were continuously fasting.

Experiment LII.—(*a*) 12-30 noon. A fowl received a subcutaneous injection of 0·006 gramme of dried venom in 1 c.c. of distilled water. Strongly-marked local muscular action occurred at the site of injection.

For a few minutes the bird sat quietly. It then became restless, stood up and made conspicuous swallowing movements.

12-35 Noon. Violent general convulsions and screaming set in and were immediately succeeded by death.

(*b*) 12-52 noon. A fowl received a subcutaneous injection of 0·006 gramme of dried venom in 3 c.c. of distilled water. The solution of venom was the same as that employed in (*a*) after dilution to the required extent.

The bird showed just the same symptoms as those in the first four experiments and then gradually recovered.

Experiment LIII.—10-35 A.M. A fowl received a subcutaneous injection of

0·0056 gramme of dried venom in 1c.c. of distilled water. Excessive local muscular action attended the injection.

For a short time the bird remained standing, and seemingly somewhat excited, judging from the jerky character of the movements which it made.

10·39 A.M. It sat down, making slight mandibular movements.

10·41 A.M. Furious general opisthotonic convulsions set in, the body revolving backwards on its transverse axis, the wings being at the same time widely expanded. Loud screaming occurred coincidently, and death took place a minute later.

Experiment LIV.—11-18 A.M. A fowl received a subcutaneous injection of 0·0056 gramme of dried venom in 1c.c. of distilled water. Well-marked local muscular action attended the injection.

11-20 A.M. The bird sat down.

11-21 A.M. Violent general convulsions set in.

11-22 A.M. Death occurred.

The record of results of this nature might be indefinitely extended, but even the data furnished by the above experiments are sufficient to indicate conclusively that in Daboia-venom we are dealing with a poison the lethal action of which is entirely distinct from that of cobra-venom. Before, however, entering into this question in detail, it may be well to consider the nature of the phenomena attending the cumulative action of fractional doses of Daboia-venom of ascertained lethal property, and that of those following the immediate introduction of excessive ones.

Experiment LV.—On the effects of repeated injection of 0·001 gramme of dried venom, of which 0·003 gramme in 1c.c. of water when introduced into the system at once sufficed to cause violent general convulsions and death within the course of ten minutes.

11-22 A.M. A fowl received a subcutaneous injection of 0·001 gramme of dried venom in 0·5 c. c. of distilled water. Violent local muscular action occurred at the site of injection.

11-42 A.M. No special symptoms present. A second injection of 0·001 gramme of dried venom was introduced into the same site as the former one and was attended by renewed local muscular action.

12-2 Noon. The bird was standing perfectly motionless and watchful. A third injection of 0·001 gramme of dried venom was introduced into the same site and local muscular action again manifested itself.

12-22 Noon. Sitting motionless. A renewed injection administered in the same site. Local muscular action barely perceptible.

12-45 Noon. A subcutaneous injection was introduced into the opposite thigh and was followed by vigorous local muscular action.

Three more injections were administered in the same site as the previous one. The first of them was attended by well-marked local muscular action, but in the two latter the phenomenon was almost absent.

The bird remained sitting perfectly motionless, and after a time the respiration was somewhat hurried and the beak kept permanently gaping. There were throughout no signs of the peculiar laboured gasping respiration so characteristic of cases of intoxication from cobra-venom, and the head never tended to droop forward. The symptoms continued unchanged until 3 A.M. of the following morning when death occurred.

In this case 0·008 gramme of dried venom, when cumulatively introduced entirely failed to give rise to any symptoms of centric nervous irritation, although a dose of 0·003 gramme would certainly have induced fatal general convulsions within the course of a few minutes had it been introduced at once in 1 c. c. of distilled water.

Experiment LVI.—On the effects of repeated subcutaneous injections of 0·001 of dried Daboia-venom, of which 0·006 gramme certainly caused violent general convulsions and death within the course of a few minutes when introduced at once in 1c.c. of distilled water.

11-40 A.M. A fowl received a subcutaneous injection in the right thigh of 0·001 gramme of dried venom in 1c.c. of distilled water. Well-marked local muscular action occurred.

Four more similar injections were introduced into the right thigh and four into the left one at intervals of twenty minutes. Muscular action failed to appear in either thigh after the first two injections. Soon after the beginning of the experiment the bird sat down in one corner of the cage and remained absolutely motionless, with the neck somewhat retracted and the back elevated. On the following day it remained in the same state, sitting perfectly motionless, with the eyes permanently closed, but the head showing no tendency whatever to droop forward. Blood-stained evacuations were occasionally passed. On the third day it showed signs of recovery, for, although still sitting as before, the eyes were now open and it chirped at intervals. On the next day recovery continued to progress, and the head was occasionally moved, and from this time onwards no relapse took place and in a short time health appeared to be completely restored.

Experiment LVII.—10-50. A.M. A fowl received a subcutaneous injection of 0·001 gramme of dried venom, of the same lethal quality as that employed in the previous experiment, in 1 c. c. of water in the right thigh, and, subsquently, at intervals of twenty minutes, two more similar injections in the same site. Strong local muscular action attended the first injection, but were absent after the latter ones. After another interval of twenty minutes a similar injection was introduced into the opposite thigh and was followed by well-marked local muscular action. Four more similar injections were subsequently introduced into the right thigh at intervals of twenty minutes. Local muscular action attended the first of them but was entirely absent in the subsequent ones. After the first few

injections had been administered the bird became absolutely motionless. It remained standing for a short time and then sat down, still and watchful. After sometime a tendency to permanent mandibular gaping manifested itself. On the following day it remained sitting absolutely motionless, with the neck retracted, the eyes open, and the back somewhat elevated. There were no symptoms whatever resembling the specific symptoms of chronic intoxication by cobra-venom. It remained in the same condition for the three next days, and on the morning of the following one was found dead and rigid, still retaining the same position.

The results of these and similar experiments clearly demonstrated the impossibility of inducing general convulsions and rapid death by means of cumulative administration of the venom, and that in order to the occurrence of such phenomena the latter must enter the system at once in certain quantity and with a certain degree of concentration. With the most potent samples of dried venom the introduction of 0·003 gramme sufficed certainly to induce violent general convulsions and death when introduced simultaneously and in solution in 1 c. c. of water, but a like amount was powerless to do so when in solution in 3 c. c. of water, and the same held good of the cumulative introduction of much larger quantities by means of repeated injections of fractional doses. The latter are capable of inducing phenomena of local nervous irritation followed by symtoms of local nervous exhaustion, but they fail either individually or cumulatively to give rise to any appreciable signs of centric irritation. The condition of local nervous exhaustion following the primary irritation in certain cases seems to be of a transitory nature, as in some instances in which local muscular action entirely fails to make its appearance in connection with injections of venom repeated at intervals of twenty minutes, it will again begin to manifest itself if the period of the intervals be doubled. The general phenomena attending the cumulative administration of normal Daboia-venom in doses too small or too dilute to induce violent general convulsions and death within the course of a few minutes, are of precisely the same nature as those attending the action of relatively large quantities of venom in which the nerve-irritant toxin is deficient in amount or entirely absent, as it frequently is in samples which have been kept for any length of time, and as it certainly is, as Wall first pointed out,* in specimens which have been heated to boiling. All evidences of direct centric nervous stimulation are absent and are replaced by an excessive immobility and by indications of the occurrence of blood-change, but of blood-change of a nature absolutely distinct from that attending the action of cobra-venom, and manifesting itself in a great tendency to the occurrence of sanguinolent effusions, and in a peculiar brick-red colour and absence of coagulability in the blood when removed from the vessels.

* Op. cit.

During life sanguinolent evacuations very frequently are conspicuous symptoms, and on post-mortem examination sanguinolent effusions will constantly be found, not merely in the immediate neighbourhood of the site of introduction of the venom but extending thence in diffuse fashion over wide areas. In the case of fowls which die in consequence of repeated injections of venom into the subcutaneous tissue of the thighs, sero-sanguinolent effusions are ordinarily present, extending thence over the whole flanks and up as far as the origins of the wings; the phenomena being, in fact, almost precisely identical with those which attend the subcutaneous injection of relatively large quantities of virulent comma-bacilli in similar sites.

From the results of the foregoing and similar experiments it is quite clear that, whilst in the case of any sample of cobra-venom we are dealing with a material in which we can readily determine that the amount constituting a minimal lethal dose bears a simple relation to weight, in that of any sample of Daboia-venom, it has always to be taken into account that we are dealing with one in which the relation of lethality to quantity is more complicated, not merely because of the presence of two distinct toxic principles, but because the proportions which these bear to one another may vary very considerably, and because the action of one of them is liable to be affected by the manner in which it enters the system. In the case of any sample of cobra-venom we have only to ascertain the weight constituting a minimal lethal dose; in that of Daboia-venom we have to ascertain the weights containing the minimal lethal doses of two perfectly distinct toxic principles, and, further, under what conditions one of the latter is capable of producing its maximal effects. Having once ascertained that a given amount of cobra-venom is lethal we know that, however it be introduced into the system, it will cause death; in the case of Daboia-venom we have in the first place to ascertain what amounts of it contain lethal doses of two distinct toxins, and then, in regard to one of these, to determine how it must be introduced into the system to produce its full effects.

It now remains to consider the phenomena attending the immediate introduction of relatively large doses of the venom into the system as compared with those which have been previously shown to follow the introduction of large doses of cobra-venom.

Experiment LVIII.—10-44 A.M. A fowl received a subcutaneous injection of 0·01 gramme of dried venom (of which 0·003 was sufficient to cause general convulsions and death within ten minutes when administered in 1 c.c. of water) in 1 c.c. of water.

Strong local muscular action attended the injection and the bird presently sat down, making conspicuous swallowing movements once or twice.

10-47 A.M. It began suddenly to jerk its head about from side to side and then at once passed into furious general convulsions, shrieking loudly, and with widely expanded and fluttering wings.

10-48 A.M. Dead. Comb and wattles bright red. Death occurred in tetanic spasm with the wings widely expanded and curved, and the legs rigidly extended, and this position was permanently retained.

Experiment LIX.—11-10 A.M. A fowl was bitten in the thigh by a large, fresh Daboia.

11-12 A.M. Violent general convulsions set in.

11-13 A.M. Dead.

Experiment LX.—10-18 A.M. A fowl was bitten by a large, fresh Daboia. It at first appeared to be restless and excited, jerking its head about in a peculiar way.

10-19 A.M. Furious general convulsions set in and half a minute later death occurred.

The next two experiments illustrate the effects following the bite of a Daboia as compared with those attending the bite of a cobra.

Experiment LXI.—(*a*) 10-18 A.M. A fowl was bitten in the thigh by a large, fresh Daboia.

It remained at first standing motionless and making swallowing movements.

10-19 A.M. It suddenly staggered backwards and passed into furious opisthotonic convulsions.

10-20. A.M. Dead.

(*b*) 10-37 A.M. A fowl was bitten in the thigh by a large, fresh cobra.

It remained standing on the uninjured leg for a short time and then sat down.

10-44 A.M. Beginning to gape and to inspire gaspingly.

10-45 A.M. The head beginning alternately to droop forward and to be elevated suddenly with each inspiration.

10-46 A.M. The head permanently depressed with the tip of the beak resting on the floor of the cage.

10-47 A.M. Asphyxial convulsions setting in.

10-54 A.M. Dead.

Experiment LXII.—(*a*) 11-10 A.M. A fowl was bitten in the thigh by a large, fresh Daboia, but the venom of only one fang entered the tissues.

11-12 A.M. Violent general convulsions set in.

11-13 A.M. Dead.

(*b*) 11-31 A.M. A fowl was bitten in the thigh by a large, fresh cobra.

It remained at first standing on the uninjured leg, the tips of the toes of the other one only touching the floor of the cage.

11-33 A.M. It sat down with the eyes closing, and the mandibles gaping with each laboured, gasping inspiration. The head gradually drooped further and further forward between each inspiration until the tip of the beak rested on the floor. Gradually the head fell over to one side and the bird lay flat with extended neck, save when it occasionally roused itself and suddenly threw up the head during inspiration.

11-36 A.M. General convulsions set in and recurred at short intervals.

11-41 A.M. Dead.

The following experiment illustrates the phenomena attending the direct introduction of minimal lethal doses, of Daboia-venom on the one hand and of cobra-venom on the other, into the circulation.

Experiment LXIII.—(*a*) A sample of Daboia-venom was carefully tested and the minimal lethal dose requisite to occasion general convulsions and death ascertained to amount to between 0·005 and 0·004 gramme.

11-7 A.M. A fowl received an intrajugular injection of 0·005 gramme in 0·5 c.c. of distilled water.

Furious general convulsions set in before the bird could be put down and death occurred immediately afterwards. The blood was of a dull brick-red colour. It brightened in some degree on exposure to air, but was absolutely incoagulable.

(*b*) 10-50 A.M. A fowl received an intrajugular injection of 0·0005 gramme of dried cobra-venom, of which that amount constituted a minimal lethal dose.

No appreciable symptoms whatever manifested themselves until between 2 and 3 P.M., but they then began to appear, ran their normal course and terminated in death between 7 and 8 P.M.

The phenomena which attend the mixture of various amounts of Daboia-venom with blood outside the body are also, as the following experimental data clearly show, quite district from those which attending the action of cobra-venom.

Experiment LXIV.—A covered glass capsule having been carefully sterilised, a solution of 0·003 gramme of dried Daboia-venom of ascertainedly highly lethal property, in 1 c. c. of sterilised distilled water, was introduced into it, and 19·69 grammes of blood added directly from the throat of a fowl at 11 A.M.

The blood at once became of a vivid scarlet colour and rapidly underwent coagulation, but the clot was persistently of an extremely soft, tremulous consistence.

11-30 A.M. The blood consisted of a relatively small clot, vivid scarlet superficially and darker red in substance, floating free in a large bulk of brilliant scarlet opaque serum.

12 Noon. The serum had now deposited a scarlet sediment of blood corpuscles and was of a muddy colour.

2 P.M. The clot was relatively very small, widely separated from the sides of the vessel and floating free in the muddy serum. The deposit had now lost its scarlet colour but portions of it, when exposed to the air, rapidly re-gained it. There was no appreciable evidence of any solution of corpuscles.

On the following day the clot was small and of a very dark plum colour, and 11 c.c. of very dark, opaque plum-coloured serum surrounded it. Both

clot and serum brightened considerably in colour on exposure to air. Many red corpuscles were still well preserved.

Experiment LXV.—This was conducted in precisely the same fashion as the previous one, 31 grammes of blood being added to a solution of 0·001 gramme of dried venom in 1 c. c. of distilled water at 11-25 A.M. The blood very rapidly coagulated into a loose, tremulous, vivid-scarlet clot.

11-40 A.M. A large quantity of bright scarlet, opaque serum had already separated.

11-55 A.M. The clot was vivid scarlet on the surface and dull scarlet in substance.

1·15 P.M. The preparation now consisted of a large quantity of muddy serum with a red deposit of corpuscles, and a relatively small clot floating free in it. The clot where exposed to the air was bright scarlet, and where submerged plum-coloured.

Twenty-four hours after the beginning of the experiment the clot was widely shrunken away from the sides of the vessel and, where not submerged, of a dull red colour. It floated in 15 c. c. of dark plum-coloured serum, which on exposure to the air in thin layers became bright brick-red. The surfaces of the clot also on exposure to air brightened to a dull scarlet. Many red corpuscles remained still intact.

Experiment LXVI.—Two capsules, *a* and *b*, were sterilised as usual. A solution of 0·01 gramme of dried Daboia-venom in 1 c.c. of distilled water and 23·69 grammes of blood were introduced into *a* at 10-40 A.M., and a solution of 0·01 gramme of dried cobra-venom in 1 c.c. of distilled water and 22·59 grammes of blood into *b* at 10-42 A.M.

a. The blood very rapidly formed a very loose, soft, tremulous coagulum of a vivid scarlet colour. The clot retained its soft consistence until the exudation of serum began to take place shortly after 11 A.M.

11-23 A.M. The blood now consisted of a soft clot floating almost free in a large quantity of opaque, scarlet serum. The surface of the clot was vivid scarlet and the sides of it bright plum-coloured.

12 Noon. The corpurcles in the serum had now subsided to form a scarlet deposit beneath a muddy fluid. The clot was now widely shrunken from the sides of the vessel and its surface was still bright scarlet.

2 P.M. The surface of the clot, which now floated free in a large quantity of muddy serum, was still bright scarlet and there was no appreciable evidence of any solution of red corpuscles.

On the following day the surface of the clot was plum-coloured mottled with deep red, and the substance plum-coloured. It floated in 13 c. c. of opaque, muddy, plum-coloured serum. On exposure to air the surfaces of the clot brightened to a dull red. The red corpuscles were apparently thinned and wanting in substance, but a very large number of them were still readily recognisable.

b. The blood rapidly formed an even, firm, coagulum the surface of which soon became bright scarlet but presently after wards began to darken rapidly.

11-23 A.M. The surface of the coagulum was dark red and its substance deep plumcoloured. No free serum was yet present.

1 P.M. The surface and substance of the coagulum were almost black, from the intense darkness of the red colour which they possessed. There was a little, limpid, dark-red serum present, but the clot was every where closely adherent to the sides of the vessel.

2 P.M. The characters of the specimen remained as before. The bodies of the red corpuscles were almost universally dissolved, leaving their nuclei free.

On the following day the clot was throughout of such an intensely deep lake colour as to be apparently black, and was everywhere closely in contact with the sides of the vessel. Only 1 c. c. of serum was present, transparent and of a deep lake colour in thin layers but in mass appearing black and opaque, only the nuclei of the red corpuscles remained recognisable.

The above experimental data are surely sufficient to demonstrate that Daboia-venom is entirely unlike cobra-venom in its nature and action and that the two poisons differ from one another in the following respects :—

1. The minimal lethal dose of normal cobra-venom is very much smaller than that of Daboia-venom.

2. But a minimal lethal dose of normal, potent Daboia-venom is much more rapidly fatal than a minimal lethal dose of the most potent cobra-venom.

3. The manifestation of the action of minimal lethal doses of normal cobra-venom, even when directly introduced into the circulation, is preceded by a prolonged latent period which lasts for some hours, but the symptoms of the action of minimal lethal doses of Daboia-venom of normal quality make their appearance in any case very rapidly, and in cases of direct introduction into the blood-vascular system, are developed instantaneously. In other words, the phenomena attending the action of minimal lethal doses of cobra-venom are essentially those of the action of a ferment-poison whilst the symptoms following corresponding doses of normal Daboia-venom are those characteristic of the action of direct nervous irritants such as strychnia.

4. Normal Daboia-venom gives rise to well-marked local muscular action at the site of introduction, but normal cobra-venom does not do so. In some exceptional cases, however, a certain amount of local muscular action follows the subcutaneous injection of solutions of cobra-venom, so that it appears to be possible that the nerve-irritant constituent which is the cause of death in acute intoxication by Daboia-venom may be a normal constituent of cobra-venom also. Even if it be, however, the maximal amount of it which is present is too small to

give rise to any thing save local effects, and, hence, in so far as the lethal properties of the venom are concerned, it is of no practical importance.

5. The phenomena attending the administration of sub-lethal doses so long as these are capable of producing any appreciable effects, of minimal lethal doses, and of excessive doses of cobra-venom are essentially alike, but in the case of normal Daboia-venom this is not the case, as sub-lethal doses, where they produce any appreciable effect, are followed by symptoms indicative of the action of one toxic principle, and minimal lethal and excessive doses by symptoms indicative of that of another.

6. In the phenomena normally attending the action of cobra-venom there is no distinct evidence of the action of anything but a single toxin, whilst in the case of Daboia-venom there is the most unequivocal evidence of the presence of two distinct lethal toxins, one acting as a direct nervous irritant and the other giving rise to septic changes in the blood.

7. The toxin in cobra-venom acts cumulatively; in the case of Daboia-venom the toxin which causes nervous irritation cannot act cumulatively whilst that which induces septic phenomena does so.

8. Dilution of minimal lethal doses of cobra-venom in no way affects their potency, whilst dilution of minimal lethal doses of normal Daboia-venom ordinarily renders them incapable of causing death because it renders the nervous irritant toxin incapable of acting upon the nervous centres, whilst the septic one, as a rule, is only lethal when it enters the system in relatively large amounts.

9. The symptoms attending the action of the septic factor in Daboia-venom, although it is capable of acting cumulatively and is apparently unaffected by dilution, are perfectly distinct from those attending the action of cobra-venom, for, whilst in cases of intoxication by cobra-venom respiratory and nervous symptoms indicative of defective oxygen-supply constitute the characteristic phenomena, in cases of chronic Daboia-poisoning such phenomena are entirely absent and are replaced by symptoms indicative of a tendency to abnormal escape of blood-constituents from the blood-vascular system. Farther, in the case of death from the action of cobra-venom the blood is characterised by its intensely dark colour and by the fact that it coagulates rapidly and firmly unless excessive doses of venom have been directly introduced into the system, or life has been artificially prolonged so as to give the ferment-action full time to operate; whereas in the case of death from the action of Daboia-venom it is of a peculiar dull brick-red colour and normally quite incoagulable, even when death has been induced almost instantaneously by the direct intravenous injection of minimal lethal doses.

There are certain points in connection with the action of Daboia-venom which appear to merit special consideration. These are, first, the phenomena

attending the dilution of minimal lethal doses of the constituent which acts as a nervous irritant; second, the fact that in certain cases the blood retains its coagulability after death; and, third, that the properties of the venom appear normally to vary at different times of year.

The only apparent explanation of the phenomena attending dilution of minimal lethal doses is that the material which acts as a direct nervous irritant is either very rapidly excreted or very rapidly decomposed, so that in the case of minimal lethal doses a delay in absorption may prevent the accumulation of enough of it within the circulation to occasion fatal irritation of the nervous centres. When a minimal lethal dose is subcutaneously injected in 3 in place, of 1 *c. c.* of water it may be assumed that, other things being alike, the absorption of the total mass of venom will be proportionately delayed and, with this, the time for its partial excretion or decomposition proportionately increased. Whether the phenomenon is actually dependent on very rapid excretion or very rapid decomposition must in the meantime remain an open question, but it appears not to be inprobable that decomposition is the essential factor, seeing that there are various facts which clearly indicate an extreme instability in the constitution of the material. Dr. Wall's experiments unequivocally showed that an exposure to temperatures which in no way affected the septic potency of the venom was sufficient to render it entirely inert as a nervous irritant. * Indeed, as he was unaware that in any case the septic phenomena may be induced without the induction of any save purely local nervous irritation by means of the cumulant employment of minute doses, he regarded preliminary heating of the venom as an essential step towards their study uncomplicated by the others. But it is not merely exposure to excessive temperatures which alters the properties of the venom in this way; for, during the moist, warm weather which prevails in Calcutta during the rainy season, a similar loss of nerve-irritant property almost invariably occurs in any specimens of venom which are not most carefully protected from all exposure to ordinary atmospheric air. Even when kept in carefully stoppered bottles, unless these be continuously retained in an artificially desiccated atmosphere they lose greatly in nerve irritant property, whilst retaining septic potency unimpaired. This loss of power came out very clearly in the case of a peculiarly potent sample of dried venom which had been employed in experiments during the course of the winter of 1894, and subsequently set aside in a stoppered bottle for some months. In its original state its potency was such that 0·003 gramme when administered in one 1c.c. of water invariably induced fatal general convulsions in fowls of about 1 kilo in weight within ten minutes subsequent to administration, but when it was again tested during the course of the

* Op. cit.

autumn, doses of 0·003 and 0·006 gramme failed to give rise even to any signs of local nervous irritation, and doses of at least 0·012 gramme were necessary in order to induce fatal general convulsions. The instabilty of the material is further indicated by the results which attended the following experiments on the action of the albumin and globulin which my friend, Dr. Warden, was good enough to separate from a sample of venom of ascertainedly high neurotic property, and which show that during the course of the treatment to which it had been exposed the venom had lost all power as a general nervous irritant.

Experiment LXVII.—A fowl received a subcutaneous injection of 1 c.c. of a solution of the albumin, that is, an amount equivalent to 0·01898 gramme of the original dried venom. Strong local muscular action occurred, but no symptoms of central nervous irritation supervened. The bird presently sat down and passed into the condition of watchful immobility characteristic of the action of the septic toxin, but after some time these symptoms wore off and recovery occurred.

Experiment LXVIII.—A fowl received injections of 2 c.c. of the same solution of the albumin which was employed in the previous experiment with similar results.

Experiment LXIX.—A fowl received a subcutaneous injection of 1 c.c. of solution of the globulin of the same strength as the solution of the albumin employed in the previous experiments.

No local or general symptoms indicative of the action of either toxic principle of the venom occurred.

It is more difficult to find an explanation for the variability in the phenomena presented by the blood in respect to coagulability in cases of death caused by the action of the poison. In almost all cases a condition of absolute incoagulability is present, but now and then an exception occurs in which the blood although presenting the peculiar and characteristic brick-red colour, associated with the action of the poison, coagulates slowly and partially. Wall affirms that this retention of coagulability only presents itself "where an animal dies almost instantaneously from the convulsions, and also when it dies after a very long interval from exhaustion."* Had it been an invariable rule that coagulability should be present in such cases the phenomenon might have been simply accounted for on the assumption that the loss of coagulability was entirely owing to the action of the septic factor in the venom. On this theory the occurrence of coagulation in cases of almost instantaneous death might be credited to the absence of sufficient time for the induction of any important changes in the blood, and the parallel phenomenon in cases where death is very greatly delayed to the occurrence of partial recovery of normal properties in it. It would, however, at first sight appear to be a fatal objection to any such explanation that even in

* Op. cit.

cases, such as that of Experiment LXIII, in which death occurs immediately in consequence of intravenous injections of minimal lethal doses of venom, evidence of profound blood-change in the form of the characteristic alteration in colour and absolute incoagulability may be present. But it must be taken into account that in such experiments the poison enters the blood directly and that this may have an important modifying effect on the results, for, if the nerve-irritant toxin be more readily absorbed than the septic one, it is evident that under normal circumstances a lethal amount of the former may have entered the blood at a time when comparatively little of the latter has been able to do so; whereas in cases of direct intravenous injections the full amount of both toxins present in the dose of venom enter the circulation simultaneously, so that, although death is almost immediately induced by the action of the nerve-irritant one, the septic one has been present in sufficient amount to induce considerable blood-change ere its supervention. Taking every thing into account, then, it appears not to be improbable that the factor in the venom which acts as a nervous irritant is incapable of inducing any important changes in the blood, and that the variability in the evidence of the occurrence of such changes in different instances is connected with variations in the amount of the septic factor which has entered the blood and in the time which has been given to it to produce its maximal effects there.

The occurrence of seasonal variations in potency is a phenomenon which is not peculiar to Daboia-venom for we find evidence of it in the case of cobra-venom also, but the results are more striking in the former case owing to the fact that, whilst immediately sub-lethal doses of cobra-venom cause profound intoxication, corresponding doses of normal Daboia-venom do not do so because the amount of the septic toxin normally associate with sub-lethal quantities of that which acts as a nervous irritant is too small to occasion any conspicuous effects. In the case of cobra-venom, samples collected during the hot and rainy seasons appear, as a rule, to be somewhat less potent than those obtained during the winter, the normal minimal lethal dose of the former for fowls of about one kilo in weight, amounting to 0·0005 gramme, whilst that of the latter is 0·00025 gramme. The same holds good in regard to Daboia-venom, the minimal dose capable of occasioning fatal general convulsions in fowls of 1 kilo in weight, amounting to about 0·003 gramme in dried samples collected during the latter part of winter, and rising to about 0·006 or even to 0·012 gramme in those obtained during the course of the hot and rainy seasons. Such variations in potency appear to occur in the venom of all snakes whether at liberty or in confinement, but are more uniform and marked in connection with the latter, for samples of venom of maximal or nearly maximal potency are occasionally obtained from freshly-caught snakes at seasons when those which have been in confinement for any considerable time are persistently yielding nothing but

weak venom. The explanation of these variations was first suggested by an old and very experienced keeper who accounted for their occurrence as the result of the fact that during the course of the cold season the snakes become very torpid and entirely cease to feed for many weeks at a time, whilst during the rest of the year they are feeding freely and therefore frequently discharging their venom. Careful observations were made in regard to this point on the Daboias in captivity in the Calcutta Zoological Garden during the course of the past year, and there could be no question of the fact that their venom became more potent shortly after they had ceased to feed in the early part of winter, and deteriorated in quality again in spring after they had again begun to feed freely. There can be no question in regard to the coincidence of the phenomena, and the fact that the seasonal variations are more constant in captive than in free snakes seems to indicate that there really is a causal connection between frequency of discharge and quality of venom. Captive snakes are certain of obtaining their food regularly, whilst those which are at large may frequently be unable to secure any prey for considerable periods, and, if they be captured towards the close of any such period, the venom which they will then yield represents the product of a continued period offasting. The phenomena are, to a certain extent, parallel to those attending the bites of fresh and exhausted snakes; only in the latter case variations in quantity as well as quality of venom probably come into play, whereas in the case of the seasonal variations the differences are dependent on variations in quality only, and would appear to indicate that the fluid, after secretion and whilst still retained within the glands, undergoes certain maturative changes in the course of which its lethal properties undergo an increase.

The results of detailed comparison of the phenomena presented by cases of intoxication by the venom of the Cobra and Daboia appear to warrant the following conclusions:—

1. The lethal properties of Cobra-venom are dependent on the presence in it of a single toxin of the nature of a ferment, which acts by inducing such changes in the blood as to render it unfit to meet the respiratory demands of the system.

2. The lethal properties of Daboia-venom are dependent on the presence of two distinct toxins, one of which is entirely devoid of any ferment-property and acts as a direct nervous irritant leading to excessive stimulation and subsequent exhaustion of the nervous apparatus, whilst the other is a material inducing septic alterations in the blood.

It now remains to be considered how far the differences in the properties of the venom of these two species of snakes may be taken as indices to the existence of corresponding differences in the properties of the venom of Colubrine and Viperine snakes generally. Unfortunately the data in regard

to this point are not of a very complete character, as opportunities of such full comparative investigation of the qualities of the venom of any other species have not presented themselves, and, indeed, it is only with the venom of one or two other species that any experiments at all have been conducted in connection with the present investigation. Some experiments have, however, been tried in regard to the action of the venom of two colubrine snakes, *Bungarus cœruleus* and *B. fasciatus*, and of one viperine one, *Echis crinata*.

Specimens of *Bungarus cœruleus* are not easy to obtain in Calcutta, and, when they are obtained, are too valuable to be subjected to the ordinary handling which has to be employed in taking venom, so that the only experiment which has been as yet tried in the Laboratory of the Zoological Garden there, was one in which an exceptionally large snake was caused to bite a fowl. The details of the results of it are given below.

Experiment LXX. 11-40 A.M.—A fowl was bitten in the thigh by a large *Bungarus cœruleus*.

The bird sat down at once, making frequent swallowing movements and closing its eyes. These symptoms were, however, evidently purely emotional as they presently disappeared and the bird stood up and began to peck about unconcernedly at objects on the floor of the cage.

11-49 A.M. It sat down again. Swallowing movements were conspicuous and the respirations deep, inspiration being occasionally accompanied by mandibular gaping. The head gradually drooped more and more forward until the tip of the beak rested on the floor of the cage.

12-5 Noon. Symptoms profound; lying quite flat with the neck extended and the head on one side; comb and wattles becoming livid.

12-15 Noon. General convulsions set in and were repeated at brief intervals.

12-27 Noon. Dead. The blood was very dark coloured and only very slowly brightened on exposure to air. It coagulated rapidly and firmly.

The symptoms throughout were practically identical with those present in acute cases of intoxication by cobra-venom. The relatively protracted duration of life subsequent to the infliction of the bite may possibly indicate that the venom of this species is not quite so potent as that of the cobra, but no definite conclusion in regard to this question can be arrived at until it has been possible to try a series of accurate experiments with ascertained weights of dried venom.

The information regarding the lethal properties of the venom of *Bungarus fasciatus* is, fortunately, somewhat more complete as the following experimental data will serve to show. The dried venom which was employed was collected from several distinct specimens of the species and in outward appearance only differed from normal specimens of dried cobra-venom in being of a somewhat paler colour than they are. The quantity which can be obtained from fresh snakes of fair average dimensions on any one occasion is normally very much

less than that yielded by cobras of corresponding size, the average weight of dried venom obtainable on each occasion being only 0·0498 gramme, whilst the average for cobras is 0·245 gramme.

Experiment LXXI.—11-42 A.M. A fowl received a subcutaneous injection of 0·001 gramme of dried venom in 1 c.c. of distilled water.

It appeared to be rather dull for some time, but no other symptoms of any kind presented themselves.

Experiment LXXII.—11-40 A.M. A fowl received an injection of 0·003 gramme of dried venom in 3 c.c. of distilled water.

No symptoms, save a certain degree of drowsiness, ensued.

Experiment LXXIII.—10-53 A.M. A fowl received a subcutaneous injection of 0·005 gramme of dried venom in 5 c.c. of distilled water.

No appreciable symptoms indicative of any specific action occurred.

Experiment LXXIV.—11-12 A.M. A fowl received a subcutaneous injection of 0·01 gramme of dried venom in 1 c.c. of distilled water.

11-50 A.M. Apparently slightly drowsy and occasionally making conspicious swallowing movements.

11-55 A.M. Inspiratory mandibular gaping beginning to appear.

12 Noon. The head beginning to droop forward so that presently, during the intervals between each inspiration, the tip of the beak rested on the floor of the cage.

12-20 Noon. The characteristic specific symptoms of intoxication by cobra-venom well developed. The eyes occasionally closing; respirations deep and laboured, with elevation of the head and mandibular gaping at each inspiration.

1 P.M. Symptoms gradually becoming more profound. The eyes permanently closed and the tip of the beak resting on the floor of the cage.

7-45 P.M. Death occurred.

Experiment LXXV—10-41 A.M. A fowl received a subcutaneous injection of 0·1 gramme of dried venom in 1 c.c. of distilled water.

11-1 A.M. Profoundly affected. The tip of the beak resting on the floor of the cage between each deep, laboured respiration. Inspiration accompanied by elevation of the head and mandibular gaping; eyes permanently closed.

11-20 A.M. General convulsions; comb and wattles extremely livid.

11-45 A.M. Dead. The heart continued acting violently for some considerable time after the cessation of respiration. The blood was at first of extremely dark colour but slowly brightened on exposure to air. It coagulated rapidly and firmly.

According to the above data the venom of *Bungarus fasciatus* would appear to be practically equivalent to very feeble cobra-venom of which the minimal lethal dose for fowls of 1 kilo in weight is approximately 0·01 gramme in place of 0·00025 or 0·0005 gramme as that of normal cobra-venom is.

In none of the cases in which smaller doses were employed did any symptoms of general blood poisoning, resembling those described by Wall as occasionally presenting themselves in those of his experiments in which death did not rapidly follow the infliction of a bite, occur. This is probably to be accounted for by the fact that Wall's experiments were conducted by means of causing specimens of *Bungarus fasciatus* to bite animals; whereas in the present series of experiments dried venom was employed. The difference in the results may therefore have been owing either to the occurrence of some change in the specific constituents of the material during the course of desiccation, or, more probably, to the fact that desiccation serves to render certain extraneous elements, which are liable to be present in the fresh venom, inert. The salivary secretion of snakes, like that of animals generally, constantly contains numerous micro-organisms of various kinds, and it is quite possible that in certain cases these may possess pathogenic properties. Where, therefore, the venom is introduced in the fresh state by means of bites, there is always a possibility that results may follow which are in no way dependent on the specific action of the essential toxins of the former. But where venom, which has been subjected to prolonged and thorough desiccation, is employed, the chances of the occurrence of any such complication must be greatly diminished. On the whole, therefore, it appears to be probable that the venom of *Bungarus fasciatus* does not in any way differ specifically from that of the cobra, but only differs in containing a smaller proportion of one and the same toxic principle.

It is quite clear that *Bungarus fasciatus*, with its weak jaw, and a comparatively small quantity of relatively feeble venom at its disposal, must be incapable of contributing to any important extent to the mortality from snake-bite. The largest amount of fresh venom which was obtainable from any specimen on any single occasion only yielded 0·102 gramme of dried material; but, as 0·01 gramme constitutes an approximately minimal lethal dose for fowls of one kilo in weight, it is extremely improbable that even this maximal yield would have been sufficient to cause death in any adult human subject.

As the species is very difficult to obtain in Calcutta, and as its small size renders the amount of venom which can be collected from any individual excessively small, in the only experiments which have as yet been tried with the venom of *Echis carinata* the venom was administered by bites. The results, however, which were obtained were sufficient to show the essential identity of the material with Daboia-venom, and, consequently, that it is quite distinct in nature from the venom of the cobra and of *Bungarus cœruleus* and *fasciatus*.

Experiment LXXVI.—10-6 A.M. A fowl weighing 1,275 grammes was bitten by an Echis.

It was seized with furious general convulsions after an interval of a minute and a half and died a minute later.

Experiment LXXVII.—10-25 A.M. A fowl was bitten by an Echis. It presently sat down with its beak partly open, and occasionally making conspicuous swallowing movements.

10-29 A.M. Violent general convulsions, accompanied by loud screaming, set in.

10-30 A.M. Dead. The blood was of a dull brick-red, rapidly brightening on exposure to air. It was absolutely incoagulable.

The symptoms of acute intoxication by this venom are clearly, then, precisely of the same nature as those induced by Daboia-venom, and, from the data which are on record in regard to the phenomena present in cases of chronic intoxication following echis-bites, there can be little doubt that the two materials are in all respects practically identical.

There is thus conclusive evidence that in two distinct genera of Colubrine snakes, *Naia* and *Bungarus*, the venom possesses essentially identical properties, and that the same holds good in regard to the venom of two Viperine genera, *Vipera* and *Echis*, but that the colubrine venom is perfectly distinct in property from the viperine one. This evidence, afforded as it is by the venom of one or two species of snakes only, is, of course, by no means conclusive, but at the same time it, at all events, suggests the probability that all snake-venoms are essentially referable to two groups, each of which includes a number of varieties differing from one another in quality but not in kind, or, in other words, distinguished merely by the quantity and not by the nature of the toxic materials which they contain. In regard to Crotalus-venom, at all events, Dr. Weir Mitchell's experimental results leave little room to doubt that it is essentially a viperine venom of the same specific nature as Daboia-venom and, like the latter, containing two distinct toxic principles, one of which acts as a local and central nervous irritant, whilst the other gives rise to septic changes in the blood.

CALCUTTA:
The 19th August 1895.

D. D. CUNNINGHAM.

On Milula, a new genus of Liliaceæ from the Eastern Himalaya.

BY

SURGEON-CAPTAIN D. PRAIN,

INDIAN MEDICAL SERVICE.

The subject of this notice is one of the interesting plants which the botanical investigation of the Eastern Himalaya, conducted during the past twenty years on behalf of the Government of Bengal by Dr. G. King, Superintendent of the Royal Botanic Garden, Calcutta, has revealed to science. It was obtained by one of Dr. King's native collectors in Chumbi, the valley that lies immediately to the east of Sikkim. Politically this valley is Tibetan; geographically it forms part of the Himalayan region, since the stream that drains it finds its way southwards through Bhutan and Assam to the Brahmaputra. Its flora, however, indicates that though so near to Sikkim, it enjoys a comparatively dry climate. This is due to the fact that in place of lying open, as does the basin of the Tista which practically constitutes Sikkim, to the moisture-laden currents that sweep up from the Bay of Bengal to precipitate on the Sikkim-Himalaya the heavy rainfall which characterises this area, the Chumbi Valley has a narrow southern outlet, and the lofty ridges that enclose it practically deprive these currents of their moisture ere they reach the depression beyond.

The facies of this singular plant, which bears out to some extent the comparative dryness of its habitat, is so completely that of an *Allium* that at first sight one feels inclined, in spite of its spicate inflorescence and its solitary bract, to treat it as the type of a somewhat aberrant section in that comprehensive genus. Both characters, repugnant though they be to our generic conception of *Allium*, might be looked upon as incidental. Already *Allium* includes species with heads of sessile florets, while in some other genera the passage from a head to a spike is of the simplest;* the difference, moreover, between one bract and two, among plants like the *Allieæ* where the bracteal variation is admittedly 2—8, is no more than arithmetical. But when we find these characters associated with such apparently essential ones as a distinctly gamophyllous perianth and a decidedly 2-seriate androecium it becomes at least inadvisable to further expand the limits of a genus already so unwieldy as *Allium*

* The genus *Acacia*, for example, amongst the *Leguminosæ*; *cfr.* Prain, in *Journal of the Bombay Natural History Society*, V, 165 (1890).

is. A secondary reason for excluding the plant from *Allium* is that the specimens show no trace of the garlic odour so characteristic, even in dried examples, of most, if not of all the species of that genus.

The localisation of the genus does not appear to be difficult; an overwhelming majority of characters indicate its tribal position to be among the *Allieæ*, (tribe xii of *Liliaceæ* in Bentham and Hooker's *Genera Plantarum*). It is true that none of the *Allieæ* hitherto described have a spicate inflorescence or a solitary bract. But it must be remembered that both characters may be merely incidental; the main point is that, like the other *Allieæ*, the plant under review *is* bracteate under the inflorescence. The *Scilleæ*, with which it agrees in the single character of a spicate inflorescence, but whose facies it does not recall, have no involving bract. The secondary question of the subordinate position of the genus presents a little difficulty. Of the already recognised subtribes it clearly approaches most nearly the *Euallieæ*, and, if it must be located in a known subgroup, it is among these that it can with the least amount of dislocation be placed. The question really turns on the relative value for purposes of classification of characters derived from the inflorescence and from the individual florets. Already the *Euallieæ* include plants with a gamophyllous perianth and plants with 2-seriate filaments, so that the characters on which the generic rank of *Milula* largely depends favour the localisation of the genus in that subtribe. At the same time it must be recollected that, whether incidental or not, the characters of a spicate inflorescence and of a solitary bract are not shared by any other member of the *Allieæ*, and it seems more natural as well as more convenient to treat *Milula* as typical of a new subtribe to be inserted among the *Allieæ* immediately before the *Euallieæ*.

The systematic definitions and descriptions required to indicate its position are as follows.

Nat. Ord. LILIACEÆ.

Tribus xii. Allieæ (*definitione parum ampliata*) Bracteæ 1—∞, flores rarissime spicati.

Subtribus 1*. *Miluleæ* (sub-trib. nova, proxima *Euallieis* anteponenda). *Bulbus tunicatus: spica in scapo simplici aphyllo terminalis. Bractea membranacea sub spica* 1; *bracteolæ* 0; *perianthium gamophyllum; andrœcium regulare; capsula loculicide dehiscens.*

Milula gen. nov.—*Bulbus* tunicatus; *folia* liguliformia; *inflorescentia* spicata, bracteâ solitariâ membranaceâ obtecta; *perianthium* gamophyllum 6-partitum lobis rotundatis; *stamina* breviter epiphylla 6, petalis opposita; *ovarium* syncarpum 3-loculare, stylo simplice filiformi, ovula in loculis singulis 2; *capsula* loculicide dehiscens, seminibus 2-3; *testa* corrugata et minutissime punctulata.

Milula spicata sp. unic. *Herba* bulbo elongato dimidio inferiore squamis (foliorum veterum reliquiis) fibrosis vestito, radicibus numerosis; *foliis* linearilanceolatis scapum fistulosum bulbo duplo longiorem aequantibus excedentibusve; *spica* cylindrica bracteâ basali spathaceâ ovato-acuminatâ inclusa, floribus viridirubescentibus parvulis plurimis campanulatis, segmentis perianthii aequalibus margine undulato-fimbriatis; *staminibus* in seriebus duabus exterioribus 3 carpellis alternantibus filamentis dimidio inferiore expansis petaloideis, interioribus 3 carpellis oppositis paullo brevioribus filamentis prorsus filiformibus, antheribus versatilibus omnibus perfectis; *ovario* subgloboso ovulis ad angulum loculi interiorem parum supra basin affixis; *capsula* globosa, ovulis in loculo quoque semper 1 nonnunquam ambobus abortis; seminibus nigris.

In HIMALAYA ORIENTALI: Chumbi, apud Do-tho, *Kingii mercenar.*!

Bulbus "prasinus" 4—7 cm. longus; squamæ fibrosæ $2\frac{1}{2}$—$3\frac{1}{2}$ cm.; radices 4—5 cm.; folia viridia 10—15 cm. longa, haec $\frac{1}{3}$—$\frac{1}{2}$ cm. lata; scapus viridis liber 6—9 cm. longus, $\frac{1}{4}$—$\frac{1}{3}$ cm. crassus; spica 2—5 cm. longa, 1 cm. diam.; bractea 3 cm. longa haec 2 cm. lata; flosculi $2\frac{1}{2}$ mm. lati, perianthio $3\frac{1}{2}$ mm. filamentis 4—5 mm. longis; ovarium $1\frac{1}{2}$ mm. diam., stylo 2 mm. longo stigmate minimo; capsula 3 mm. lata tenuis; semina $2\frac{1}{2}$ mm. longa, $1\frac{1}{2}$ mm. diam.

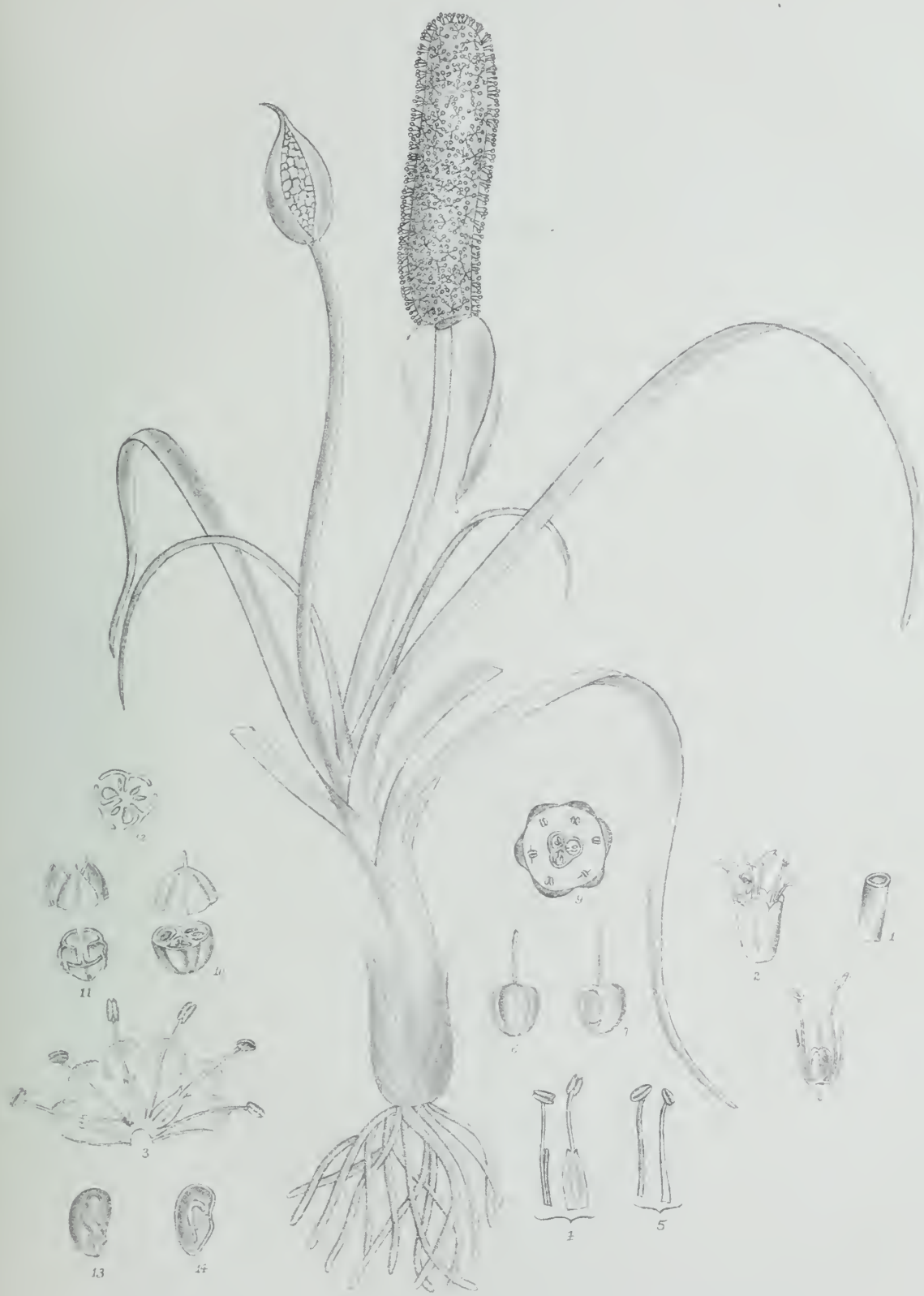
Explanation of the Plate.

. *Milula spicata*, Prain.

The specimen selected is the only one of the solitary gathering which shows 2 scapes ; the others have but one.

1. Section of stem × $\frac{1}{1}$.
2. Flower × $\frac{3}{1}$.
3. Perianth laid open to show staminal insertion × $\frac{3}{1}$.
4. Stamen of outer series from within and also sideways × $\frac{3}{1}$.
5. Stamen of inner series „ „ „ „ „ × $\frac{3}{1}$.
6. Ovary × $\frac{5}{1}$.
7. Vertical section of ovary showing an ovule × $\frac{5}{1}$.
8. Floral diagram, vertical.
9. „ „ horizontal.
10. Ripe fruit, cut across × $\frac{5}{1}$.
11. „ „ seeds fallen, showing dehiscence × $\frac{5}{1}$.
12. Diagram showing dehiscence and position of seeds and of abortive ovules.
13. Seed × $\frac{5}{1}$.
14. Vertical section of seed showing embryo × $\frac{5}{1}$.

Figs. 1 to 9 are taken from flowers belonging to the specimen delineated ; figs. 10 to 14, from fruits of a second specimen collected at the same time and place.



Drawn by D. Prain.

MILULA SPICATA, Prain.

Lith by New Cutra Sch

An experimental investigation of the effects of hæmostatic and other drugs on the intravascular coagulability of the blood.

BY

SURGEON-LIEUTENANT LEONARD ROGERS, M.B., B.S. LOND., F.R.C.S., ENG.

In December last I read a paper before the Medical Section of the Indian Medical Congress on "Some Clinical applications of the different methods of influencing intravascular coagulability," which was based on the work of Dr. Wright of Netley* and on some experiments I have been doing on the effect of various drugs on the time of coagulation. As some of the latter had been carried out under somewhat disadvantageous circumstances whilst on a march, I have since repeated and considerably extended them, and propose to set forth the results obtained in the present paper.

The method adopted is a very simple one. It consists in the determination of the coagulation-time of the blood both before and after taking a given dose of a drug by means of Dr. Wright's apparatus,† which is figured and described in the British Medical Journal, vol. I, page 237, 1894. All the precautions there advised were taken, but, during the latter experiments, the water in the vessel was slightly above "half blood heat" owing to the impossibility of getting ice in this station (Doranda). As in the different observations of any one experiment there was never a difference of more than a fraction of a degree centigrade, this could not possibly have influenced the results.

One slight modification of the apparatus was found necessary, namely, the fitting of a cork to the metal vessel containing the water, and the placing of it in a horizontal position, in order to prevent the column of blood running down and drying at the orifice of the tube. Briefly, the principle of the method is to draw up a drop of blood, obtained by pricking the finger, into a given length of uniform capillary tubes, and then by blowing down the tubes after noted intervals of time, say, at half minute intervals, to determine the period at which coagulation occurs; the shortest time in which the blood is found to be clotted so that it cannot be blown out of the tube, or, if blown out on to blotting paper, a clot is found to have been formed in it, is the coagulation-time. As

* British Medical Journal, 1893, Vol. II, pages 57 and 223.
† British Medical Journal, 1894, Vol. I, page 237.

this time varies with the temperature, as uniform a temperature as possible is advisable. "Half blood temperature," 18·5°C (65·°F) is recommended by Dr. Wright, is obtained by placing the tubes in small pockets of a bandage fitted around a metal vessel which can be filled with water of the desired temperature.

One other fallacy has to be eliminated, namely, the effect of a full meal in slightly accelerating, and of a prolonged fast in retarding the coagulability of the blood. I made some preliminary observations to determine the extent of this influence. Thus one day I had tea and toast at 7 A.M., breakfast at 9·30, and then fasted until 8 P. M., taking two-hourly observations which resulted as follows: At 9 A.M. my coagulation time was 4 min. 15 sec., at 11-30 A.M. =4 min., at 1-30 P.M.=4 min., at 3-30 P.M.=4 min., at 5-30 P.M.=from 4 min. 15 sec. to 4 min. 30 sec., at 7-30 P.M.=4 min. 15 sec., and at 10 P.M. =4 min. Thus the greatest variation was less than half a minute, and by taking a light lunch at 2 P.M. even this difference may be lessened.

Again, exercise might possibly influence the coagulation-time, but the following observation seems to show that it exerts no material effect on it. Thus a 14 mile march followed by being out shooting from 11-30 A.M. to 5-30 P.M. one day, and a 13½ mile march the next morning produced no alteration of my coagulation-time. Alcohol has been shown by Dr. Wright to retard coagulation, so throughout the 7 months over which my experiments have extended I have taken no alcoholic drinks. In fact from nearly 150 observations I have come to the conclusion that at a given temperature my coagulation-time is remarkably constant, and as no conclusions have been drawn from less than three experiments with the same drug, and from an acceleration of less than from a half to one minute, all known sources of fallacy may be fairly claimed to have been excluded.

I now pass on to the consideration in detail of the results obtained, beginning with the class of so-called hæmostatics, as the main object of this investigation was to determine in what degree, if any, the power of these drugs in checking internal hæmorrhage depends on their effect in accelerating the clotting power of the blood, the rapidity of this action, and how long it continues. As yet I have only determined this for single doses.

Calcium Chloride.

This salt is the most powerful coagulation accelerator known, as has been fully shown by Dr. Wright,* with the exception perhaps of the inhalation of carbonic acid gas.† I will give one typical experiment here which I did at Netley in 1893 to illustrate its range of action.

Experiment.—My coagulation-time having been found to be 5¼ minutes both in the morning and afternoon, I took 1 gramme (15 grains) of calcium chloride at 7 P.M., and the same dose again at 7 A.M. the next day. At 3 P.M. my

* British Medical Journal, 1893, Vol. II, page 223.
† British Medical Journal, 1894, Vol. II, page 57.

time was $1\frac{3}{4}$ minutes, a day later it was $3\frac{1}{2}$ minutes, and on the 4th day it had regained its former time of $5\frac{1}{4}$ minutes. I may just mention here that on three occasions I have seen severe hæmoptysis cease in from 10 to 20 minutes after a single gramme dose of calcium chloride, in two of which other drugs had previously failed to control it. That this very rapid effect was really due to the action of the drug and not to a series of extraordinary coincidences is borne out by the following observation. My coagulability-time being 3 minutes at 11-45 A.M. I took 1 gramme (15 grains) of calcium chloride at 12 noon. At 12-30 my time was 1 min. 30 sec. Thus in half an hour my coagulation-time was accelerated by 1 min. 30 sec., or by one-half. Calcium chloride has, however, one great disadvantage, namely, that if the dose be increased or frequently repeated, not only does its effect not increase proportionately, but is actually decreased until it has an opposite effect. It cannot then be used to keep up a definite increase of coagulability over a long period of time, as, for instance, in the treatment of aneurism.

Turpentine.

Experiment at 10-30 A.M. Coagulation-time, 4 min. 30 sec. Oil of turpentine 3ss at 10-40 A.M.

„ 12-40 „ „ „ 3 „ 15 „ or an acceleration of 1 min. 15 sec. after 2 hrs.

„ 2-40 P.M. „ „ 3 „ „ „ „ 1 min. 30 sec. after 4 hrs.

„ 4-30 „ „ „ 4 „ „ „ „ min. 30 sec. after 6 hrs.

The effect was therefore passing off in 6 hours.

On other occasions the effect was not quite so marked as in the above experiment, but the acceleration was never less than half a minute; it had nearly passed off after 12 hours, completely in 24 hours.

The good effect of turpentine in controlling internal hæmorrhages is thus in part at least due to its power of increasing the coagulating power of the blood. Its action is rapid, but also passes off quickly. As its effect is a general one on the blood, it may be used with advantage in hæmorrhages other than those from the alimentary canal as I have had occasion to verify clinically. In fact I look on it as one of our most useful hæmostatics.

Gallic and Tannic Acids.

These may be considered together, as tannic acid is said to be absorbed in the form of gallic acid.

Experiment at 11-45 A.M. Coagulation-time being 3 min. Gallic acid gr. 40 was taken at 11-50 A.M.

„ 3-50 P.M. „ „ was 2 „ 30 sec., an acceleration of 30 sec. after 4 hours.

„ 7-50 „ „ „ „ 3 „ the effect having passed off.

On another occasion an acceleration of 55 sec. followed $4\frac{1}{4}$ hours after taking 30 grains of gallic acid.

Experiment at 10-30 A.M. Coagulation-time being 4 min. Tannic acid gr. 20 was taken at 10-35 A.M.

" 1-30 P.M. " " was 3 " 35 sec., an acceleration of 25 sec. after 3 hours.

" 4-30 " " " " 3 min., an acceleration of 1 min. after 6 hours.

This effect had quite passed off by noon the next day.

Thus these drugs appear to have a distinct effect in accelerating coagulation, which takes some hours to develope and passes off fairly quickly. They are not so powerful as some of the other drugs tried. As was to be expected, tannic acid acts somewhat more slowly than gallic acid.

Persalts of Iron.

Although I cannot find any reference to the use of iron internally as a hæmostatic except in cases of hæmatemesis, etc., where its action is a local one, I was led to investigate its effect on intravascular coagulation by thinking of its powerful local action in causing blood to clot, and obtained the following interesting results :—

Experiment at 10-30 A.M. My coagulation-time being 4 min. I took liquor ferri perchloridi 3ss. at 10-35 A.M.

" 1-30 P.M. " " was 4 " 45 sec., a retardation of 45 sec. after 3 hours.

" 3-35 " " " " 2 " 15 sec., an acceleration of 1 min. 45 sec. after 5 hrs.

" 7-40 " " " " 2 " 45 sec., an acceleration of 1 min. 15 sec. after 9 hrs.

The next day " 10-30 A.M. " " " 2 " 15 sec., an acceleration of 1 min. 45 sec., after 24 hrs.

Again " 10-30 " " " being 3 " I took liquor ferri perchloridi 3i at 10-45 A.M.

" 12-45 P.M. " " " 2 " 15 sec., an acceleration of 45 sec. after 2 hours.

" 4-45 " " " " 2 " an acceleration of 1 min after 6 hours.

" 7-45 " " " " 1 " 45 sec., an acceleration of 1 min. 15 sec. after 9 hrs.

Next day at 10-50 A.M. My coagulation-time being 1 min. 30 sec., an acceleration of 1 min. 30 sec. after 24 hrs.

Again „ 10-30 „ „ „ being 3 „ I took liquor ferri pernitratis min. 40 at 10-45 A.M.

„ 1-0 P. M. „ „ was 4 min., a retardation of 1 min. after 2¼ hours.

At 3-45 P.M., my coagulation-time was 2 min. 30 sec., an acceleration of 30 sec. after 5 hours.

Next day at 10-30 A.M. „ „ „ 3 „ the effect having passed off in 24 hours.

Thus the minimum pharmacopœal doses of both salts were followed by a primary retardation of the coagulation-time, and this was quickly superseded by an accelerating effect more marked and more persistent in the case of the chloride, whilst after a one drachm dose of the perchloride an immediate gradually increasing and persistent acceleration occurred. The perchloride of iron has then a marked accelerating effect which is more lasting than any other drug I have yet tried, with the exception of calcium chloride. I have hopes that it will prove even more reliable than that salt in cases where it is wished to keep up an increase of the coagulation-power of the blood over some days as in the treatment of aneurisms, scurvy, hæmophilia, etc.

Ergotin.

Ergotin was taken in the full pharmacopœal dose of 5 grains, on three separate occasions, and observations made from two to four and a half hours afterwards. In no case was any alteration of the coagulation-time produced. Ergotin, therefore, does not act by increasing the clotting power of the blood, and as its effect in increasing arterial tension at least partly neutralises its power in contracting the arterioles, its value as a hæmostatic in hæmorrhages other than those from the uterus is, I believe, little or none.

Hamamelis.

In four experiments with the liquid extract of this drug, in doses of from fifteen (15) minims to two drachms, on only one occasion was any acceleration produced, and then of less than half a minute. The efficiency of this drug, more especially in oozing hæmorrhages, does not appear to be dependant on any effect on coagulability. According to Wood and Marshall it has absolutely no physiological action.

Dilute Sulphuric Acid.

This acid in half drachm doses produced no effect on the coagulation-time, but one drachm was followed by a slight acceleration. Thus my coagula-

tion-time being 3 min. at 10-35 A.M., I took of acid sulph. dil. 3i at 11 A.M. At 1-30 P.M. and again at 4 P.M. my coagulation-time was 2 min. 30 sec., or a quickening of 30 sec. at both 2½ and 5 hours, respectively, after this dose. Sulphuric acid has then only a slight accelerating influence when given in a full dose.

Lead Acetate.

Experiment at 10-30 A.M. My coagulation-time being 3 min. 30 sec., lead acetate gr. 5 was taken at 10-50 A.M.
at 12-50 P.M. „ „ was 2 min. 40 sec., an acceleration of 50 sec. after 2 hours.
at 3-50 P.M. „ „ was 3 min.

and at 4-45 P.M. the next day it was 2 min. 15 sec., an acceleration of 1 min. 15 sec. persisting 30 hours after taking the drug. On other occasions an acceleration of from 25 sec. to 1 min. has been observed, but the effect has in some instances passed off in 24 hours.

Thus lead acetate has a distinct and fairly persistent accelerating effect on coagulation, sufficient to account for its known value as a hæmostatic, especially in combination with other drugs.

Potassium Iodide.

This salt is not used as a hæmostatic, but as it is very frequently given for long periods in cases of aneurism, even when there is no suspicion of syphilis, I thought it would be of interest to determine if it had any effect on the coagulation of the blood. In my first experiments with this drug I obtained negative results three and four hours, respectively, after single 20-grain doses. However, on repeating the experiment, and making observations at longer intervals, I obtained the following interesting effect :—

Experiments at 10-45 P.M. My coagulation-time being 3 min., I took potassium iodide, gr. 20, at 10-50 A.M.
at 1-45 P.M. „ „ „ was 3 min. 45 sec., a retardation of 45 sec. after 3 hours.
at 3-50 P.M. „ „ „ „ 1 min. 45 sec., an acceleration of 1 min. 15 sec. after 5 hours.
at 7-20 P.M. „ „ „ was 2 min., an acceleration of 1 min. after 8½ hours.
but the next day at 10-15 A.M. „ „ „ 3 min. 20 sec., the effect having quite passed off in 24 hours.
Again at 10-15 „ „ „ being 3 min. 20 sec., I took potassium iodide, gr. 20 at 10-30 A.M.
at 1 P.M. „ „ was 2 min. 30 sec., an acceleration of 50 sec. after 2½ hours.
at 4-30 P.M. „ „ „ 2 min. 15 sec., an acceleration of 1 min. 5 sec. after 6 hours.

Thus single 20-grain doses of potassium iodide caused a marked acceleration of coagulation in from five to eight hours (in one instance preceded by a primary retardation) passing off in twenty-four hours. Its clinical use in cases of aneurisms thus receives an additional physiological basis.

In addition to the above, I have also investigated the action of some of the vegetable acids which Dr. Wright has found to diminish the coagulability of the blood.* My results are, however, much more variable than his, as will be seen from the data given below:—

Citric Acid.

Experiments (1) at 10-25 A.M.	My coagulation-time being			3 min.,	I took citric acid 3i. at 10-45 A.M.
at 12-45 P.M.	,,	,,	,,	was 3 min.,	or no change after 2 hours.
at 2-40 P.M.	,,	,,	,,	,, 2 min.,	or an acceleration of 1 min. after 4 hours.
at 4-30 P.M.	,,	,,	,,	,, 2 min. 30 sec.,	or an acceleration of 30 sec. after 6 hours.
(2) at 10-25 A.M.	,,	,,	,,	being 2 min.,	I took citric acid 5 grammes (3iiss.) at 10-45 A.M.
at 12-40 P.M.	,,	,,	,,	was 2 min.,	or no change after 2 hours.
at 2-45 P.M.	,,	,,	,,	,, 2 min. 30 sec.,	or a retardation of 30 sec. after 5 hours.
(3) at 10-20 A.M.	,,	,,	,,	being 2 min. 45 sec.,	I took citric acid 5 grammes at 10-55 A.M.
at 12-55 P.M.	,,	,,	,,	was 3 min. 15 sec.,	a retardation of 30 sec. after 2 hours.
at 3-35 P.M.	,,	,,	,,	,, 3 min. 30 sec.,	a retardation of 45 sec. after 5 hours.

Thus in one case an actual acceleration was observed, and in the others the retardation was much less marked than in the case of Dr. Wright's experiments. Possibly my citric acid, which was, however, obtained from two different sources, was not pure, but this I have no means of testing.

Tartaric Acid.

Again in the case of tartaric acid I twice observed a slight acceleration to follow 5i and 5 gramme (3iiss.) doses, respectively, and on one occasion a decrease of 1 min., $2\frac{1}{2}$ and 5 hours after a 5 gramme dose, but in this case my

* British Medical Journal, 1894 Vol. II, page 57.

coagulation-time had been previously accelerated to 1 min. 30 sec. by a dose of a persalt of iron taken the day before, so the decrease may have been due to the effect of the iron passing off. Here again my tartaric acid, which was obtained from a Calcutta chemist, may possibly not have been pure.

Lime-Juice.

Lime-juice on the other hand, when taken in one ounce doses, has uniformly been followed by a marked retardation of coagulation.

Experiment at 10-30 A.M.	My coagulation-time being 3 min. 30 sec., I took lime-juice ʒi. at 10-40 A.M.			
at 12-40 P.M.	„	„	„	was 5 min., or a retardation of 1 min. 30 sec. after 2 hours.
at 3-30 P.M.	„	„	„	was 4 min., a retardation of 30 sec. after 5 hours.

On other occasions a retardation of 1 min. 45 sec. and 1 min., respectively, was noted 2 hours after taking one ounce of lime-juice, but the effect had in one instance passed off again in 4 hours.

As the Board of Trade standard lime-juice only contains 30 grains (or 2 grammes) of citric acid to the ounce, this vegetable acid seems to have a greater power of diminishing coagulability when given in the form of lime-juice than as the pharmacopœal drug, a point I am unable to account for unless it is due to the alcohol which is added to some brands, as, for instance, that used in the navy. This fact should be borne in mind in treating cases of scurvy with a marked tendency to hæmorrhages, and same coagulative accelerator given before the lime-juice, of which I would suggest the perchloride of iron as the most suitable, or sodium citrate substituted for the lime-juice as first suggested by Dr. Wright.*

This concludes the list of drugs I have up to the present time experimented with. The clinical bearing of many of the observations will be at once obvious, but these I fully entered into in my paper read before the Indian Medical Congress, and this is not the place to dilate on them. I hope, however, to be able to give the results of further experiments on the effects of repeated doses of single and combined drugs on intravascular coagulability in a future paper.

* British Medical Journal, 1894, Vol. II, page 57.

Is Mycetoma primarily owing to the action of the fungal elements ordinarily associated with its products?

BY

BRIGADE-SURGEON LIEUTENANT-COLONEL D. D. CUNNINGHAM, C.I.E., F.R.S.

To most pathologists it will probably appear to be very heterodox to retain any doubts in regard to the relation which the presence of the fungal elements in the morbid products of the disease bear to Mycetoma. The long recognised association of masses of large fungal elements with the black concretions of the one form of the disease and Mr. Kanthack's recent demonstration of the presence of actinomyces-like structures in the roe-like bodies of the other* will probably appear to most pathologists to be conclusive evidence that these constitute the essential and primary contagium vivum on which the affection is dependent. There appear, however, to be certain phenomena which still render this doubtful, and it is to these that I propose to call attention in the following pages. They are the following: 1st, that a certain number of cases occur in which, whilst all the other essential symptoms of the disease are present, there is an entire absence of any concretions of either the black or white variety and of the fungal elements ordinarily associated with them; 2nd, that the fungal elements which ordinarily occur in connection with the two varieties of the disease are of absolutely unlike character, those associated with the white variety resembling those of actinomyces, and those of the black variety forming sclerotia like those of the Sclerotiniæ and other allied ascomycetes; 3rd, that this very formation of sclerotioid bodies is a process which is normally indicative of a cessation of vegetative growth dependent on exhaustion of nutritive supply, and therefore one which could not occur were the normal tissues the proper site for the development of the parasitic elements. Were the normal tissues the primary site in which the latter establish themselves, there could be no reason for a cessation of the indefinite extension of mycelium and for the formation of dense sclerotioid aggregates until they had been throughout affected and exhausted, whereas, as a matter of fact, we find the sclerotia isolated and surrounded by masses of tissue entirely devoid of fungal elements.

Taking these points in detail, we have first to consider the question of the possible existence of the disease without the presence of any of the fungiferous

* Madura disease (mycetoma) and actinomycosis. A. A. Kanthack, Journal of Pathology and Bacteriology, October 1892.

concretions ordinarily associated with it. Of course, on the presumption that the fungal elements are the primary and essential cause, this is a question which is not open to discussion, but, when cases of disease are encountered which both clinically and structurally are essentially identical with typical cases of mycetoma, save as regards an entire absence of concretions and fungal elements, it is one which naturally suggests itself to any unprejudiced mind and which must be determined by the nature of the evidence adduced in regard to the general identity of the two forms of disease and the absence of fungal elements in one of them.

I have, however, as I believe, met with two cases in which both of these points were quite unequivocally demonstrable. They both occurred in patients who were admitted into the Medical College Hospital, Calcutta; in both cases the disease occurred in the foot; and in both the specimens reached me in the fresh condition immediately after they had been removed by amputation. In both cases the foot presented all the characteristic external appearances of mycetoma, being thickened and shortened, with extreme flattening of the plantar arch and with numerous mammilations of the surface, which in some instances presented orifices leading into the substance of the tissues. Sections revealed the usual and characteristic system of cyst-like cavities and intercommunicating sinuses, but the contents of these consisted entirely of fluid mucoid or oily materials, and, in spite of the closest scrutiny, failed to shew any traces whatever of the presence of concretions of any kind. A specially strict search for such bodies was made, as the specimens had been procured in the fresh state for the special purpose of obtaining materials for attempted cultures of the fungal elements, but all attempts to detect any proved entirely futile.

Here then were instances of a disease in which the lesions were precisely of the same character as those which are ordinarily associated with the presence of fungal concretions, and which clinically were regarded as cases of mycetoma, but which had seemingly arisen quite apart from the presence of any fungal elements, or, at all events, from the presence of any hyphomycete fungi. It thus became necessary to look for some other cause accounting for the morbid phenomena, and in doing so a very extensive series of preparations was made which certainly revealed the presence of no filamentous fungi, but showed clear evidence of the existence of widely diffused textural changes.

The very peculiar character of the contents of the cavities and channels and, specially, the conspicuous evidences of the abundance of fungal elements in the concretions of the black variety of the disease, would appear in great measure to have diverted the attention of most observers from the study of the phenomena presented by the surrounding tissues. The accounts of these phenomena which have been recorded are extremely meagre and appear to leave one very important point entirely out of consideration. This is the evidence of very extensively diffused vascular disease of the nature of arteritis obliterans

affecting the capillaries and smaller arteries and making its appearance as the primary evidence of disease in areas which otherwise retain perfectly normal characters (Plates I and II). This phenomenon is common to both those forms of the disease in which concretions occur and to that form in which they are absent, and taking this into account along with the identity of the clinical and coarser structural features presented by all three forms of disease, I can see no satisfactory ground for refusing to recognise the third as the outcome of the same morbid process to which the other two are due, so long as the latter are regarded as mere varieties of one another. The nature of the concretions and of the fungal elements associated with them, which are characteristic of the two ordinarily recognised varieties, is absolutely unlike, and, if this is not to be regarded as indicative of anything but mere variability in what is primarily and essentially a single morbid process, there can be no satisfactory reason for refusing to admit the existence of a third variety in which concretions are absent, so long as the remaining features of the disease are essentially identical.

Allowing this, we have to deal with a morbid process which manifests itself in three distinct forms, two of which are characterised by the presence of two distinct forms of filamentous fungal elements in their products, whilst the third is distinguished by their entire absence, and, this being so, it is clear that the fungal elements cannot be the primary cause of the disease, and that we must look to something common to all three varieties as a guide to its true etiology.

Proceeding next to the question of the specific identity or distinctness of the fungal elements which constitute such characteristic features in the morbid products of the two ordinarily recognised varieties of the disease, it must be noted in the first place that Mr. Kanthack is the only observer who maintains their identity on any grounds worthy of consideration, as Carter's assumption that the concretions of the white variety are the results of degeneration of those present in the black one is based on no evidence whatever save the general resemblance which the two diseases otherwise present. In other cases in which the fungal elements are described, the descriptions generally refer to those characteristic of the black variety only, and agree in describing them as consisting of mycelium of higher fungal type, and where those occurring in the white variety are referred to they are described as presenting quite distinct characters. In his very interesting paper on the subject Mr. Kanthack allows that, unless the fungal elements proper to the two ordinarily recognised varieties of the disease can be shown to be of like nature, it must remain improbable that they constitute the primary cause of it, and it is only because he believes that he has demonstrated their identity, or that at utmost they are mere varieties of one and the same species, that he regards the affection as of primary fungal origin dependent on the invasion of the tissues by an organism which he proposes to denominate *Oospora indica*.

Now in regard to this, it must always be carefully kept in mind that Oospora is not a term of truly generic value at all, but a mere provisional one applied to a number of fungi which have not yet been definitely classified, because we are as yet only very imperfectly acquainted with their true nature. In many cases the mycelium alone has been as yet detected, and in those cases in which any fructification is present, it invariably consists of conidia or possibly in some cases of chlamydospores, neither of which are of determinative value in questions of classification. When, therefore, we describe any fungal growth as an Oospora, we do not by any means imply that it necessarily has any really close affinity to any of the other fungi included in the same group with it. All that we mean is that it is a fungal organism whose precise nature we are not at present in a position to determine. But, if this be so, we are most assuredly not in a position to determine questions of the specific identity of the different forms which, as a matter of convenience, are included within the provisional genus. Even in those cases in which fructification is present, this still holds good, for the forms of fructification which occur are not of determinative value. This must at once be evident to every one when it is taken into account that the conidial fructification, which used formerly to be described as *Botrytis cinerea* is seemingly common to many distinct species of Sclerotinia. But in the case of the fungal organisms associated with mycetoma we have no fructification of any kind to aid us, and hence any satisfactory identification becomes even more impossible. No one would dream of affirming that any two specimens of Zygomycete mycelium belonged to one and the same species of fungus, however identical in character they might be, in the absence of any fructification, and when we have not only no fructification as a guide, but there is in addition no resemblance, but very conspicuous differences between two mycelia, as is the case with the two varieties of mycetoma, in place of having any satisfactory ground for affirming that they are specifically identical, we have very good ground for suspecting that they are specifically distinct.

The mycelium discovered by Mr. Kanthack in the concretions of the white variety of mycetoma is practically identical in characters with that occurring in actinomycosis, but this most assuredly is not the case with regard to that which is normally associated with the black variety. Mr. Kanthack apparently encountered a specimen in which the fungal elements, or some of them at all events, did resemble those normal to the white variety, but this must not be taken as indicating that as a rule they do so. On the contrary, all the specimens of the black variety which have been studied by other observers from the days of Carter to those of Boyce and Surveyor are described as containing a mycelium presenting no resemblance whatever to that characteristic of actinomyces, which is characterised by the absence or extreme

rarity of septation, and presents the characters which led F. Cohn to regard his *Streptothrix* as a schizomycete organism. It is true that Sauvageau and Radais* have apparently unequivocally demonstrated that he was wrong in doing so, and that his Streptothrix is in reality a filamentous mycelium which may at present be included within the provisional group Oospora, but this does not in any way affect the question, seeing that, according to them, the species of Oospora associated with actinomycosis is characterised by an entire absence of septation. The observations of these authors alone are amply sufficient to demonstrate the purely provisional character of the genus Oospora, seeing that they make it include organisms with both septate and aseptate mycelium, and therefore presumably belonging both to the group of higher fungi and to the phycomycetes. A glance at the first figure in Plate II, which is taken from an organism occurring in a neutral salt-solution as the result of air-contamination, will show the characters presented by an Oosporic mycelium of the type of Cohn's Streptothrix, and on comparing them with figures 2-4 of the same plate, illustrating the fungal elements normal to the black variety of mycetoma, it will be at once evident that the latter are of an entirely different type. In place of being aseptate the filaments are here built up of series of very short, relatively broad cells, and in place of presenting any resemblance to those of any filamentous schizomycete, they resemble those which enter into the constitution of the sclerotia of any of the higher fungi which produce such bodies (Plate II, Fig. 3).

Whilst then, in the absence of any demonstration of definitive fructification, the fungal elements characteristic of the two common varieties of mycetoma may, as a matter of convenience, be referred to the provisional genus Oospora, this does not imply that they are specifically identical or even related to one other in any way. On the contrary, in so far as mycelial characters go, the evidence goes to show that they are absolutely unlike. The mycological evidence, such as it is, goes to show that these two varieties of the disease are associated with organisms of totally distinct nature, and this being so, it can hardly be reasonably maintained that they constitute the specific primary cause of it. The general clinical features of the disease in the two varieties, and the structural details apart from the character of the concretions, are so precisely alike that it is hard to follow observers like Boyce and Surveyor,† who whilst affirming the absolute distinctness of the fungal elements present in them, yet affirm that the latter are both pathogenic and both the specific cause of the disease with which they are associated. In other words, they affirm that two forms of disease which, in so far as all their general features

* Sur les genus Cladothrix, Streptothrix, Actinomyces, etc. Annales de l' Institut Pasteur, 1892, p. 242.

† Upon the existence of more than one fungus in Madura disease. Progs., Royal Society, June 9th, 1893.

are concerned, are practically identical, are due to the action of two distinct fungal organisms which are not even remotely related to one another!

But if there be no satisfactory ground for believing that the fungal elements of the two commonly recognised varieties of the disease are in any way related to one another, and if a third variety is present in which fungal elements are entirely absent, surely the evidence for the primary fungal origin of the affection is hopelessly defective. Even in Mr. Kanthack's specimens of the white variety of the disease fungal elements were absent from some of the concretions. Being convinced, as he was, of the identity of the fungi in both ordinarily recognised varieties, he not unnaturally ascribed this to antecedent degeneration of fungal elements which had been previously present, but such an explanation is not applicable to cases where the disease is present and fungal elements are entirely absent. When it is taken into account that the fungal elements associated with the disease are of two perfectly distinct types, that those present in the black variety constitute sclerotioid masses, that fungal elements are not invariably present in the concretions of the white variety, and, finally, that a third variety exists in which concretions and fungal elements are alike absent, can it be logically maintained that the disease has a primarily hyphomycete origin? It may be primarily of parasitic origin, but there is assuredly nothing satisfactorily to demonstrate that the recognised forms of fungal elements usually associated with it play the part of specific primary causes.

Judging from the appearances present in a very large series of preparations, by the generally accepted fact of the essential differences in the characters of the concretions present in the two commonly recognised varieties of the disease, and by the entire absence of such bodies in what there appear to be good grounds for recognising as a third variety, I believe that the evidence points to the following sequence of morbid processes in the development of the disease. At the outset we have to deal with morbid changes which are confined to the smaller arterioles and capillaries and which lead on to more or less complete obliteration of their lumina. In the capillaries an excessive proliferation of endothelium occurs, so that in stained preparations the course of individual branches may be traced almost as distinctly as though they had been injected owing to the accumulations of nuclei which they come to contain (Plate I, Figs. 1, 2, 5; Plate II, Fig. 6). As the proliferation of endothelial elements advances the cells no longer form a single layer, but become stratified, and ultimately form a plug which obstructs the lumen of the vessel and converts the latter into a solid cord. In the case of the arterioles similar processes go on and at the same time the muscular coat becomes abnormally thickened, whilst, with less uniformity and in minor degree, a certain amount of hypertrophy also takes place in the connective tissue of the outer coat (Plate I, Figs. 3, 6; Plate II, Figs. 5-7). Whilst these primary changes are advancing in the vessels other morbid phenomena begin

to make their appearance in their immediate neighbourhood. Accumulations of granulomatous tissue, frequently containing bodies similar to those which occur so abundantly in the morbid tissue in Rhinoscleroma (Plate I, Fig. 8), are gradually formed at points along their course, in some cases forming more or less continuous sheaths and in others localised aggregates, which tend specially to make their appearance at points of bifurcation (Plate II, Figs 8-11). As time goes on these accumulations steadily increase in bulk, and as they do so, and as the normal formed elements of the adjoining tissues undergo progressive solution, large areas come to be occupied by morbid vessels embedded in granulomatous tissue laid down in an intricate system of masses and intercommunicating bands. From the nature of the changes affecting the vessels, it is clear that the nutritional requirements of the morbid tissue must be very imperfectly met, and that the latter must consequently be specially liable to degeneration and necrosis. Such processes must almost necessarily be attended by reactive inflammatory processes of like distribution, and ultimately leading to the development of a ramified system of spaces and channels bounded by more or less defined walls of fibrous connective tissue and occupied by degenerate and necrosed products in various stages of alteration. The precise nature of the changes ultimately taking place in such products will naturally, not necessarily, be identical in all instances. In certain cases processes of solution into oily or mucoid matter may occur followed by absorption or caseation, or by external discharge where a communication with the surface is established. Where the morbid products are discharged, little, save persistent elastic elements, may remain behind (Plate I, Fig. 7), but where caseation takes place its ultimate products may remain for long in a passive condition. Whatever course the products pursue, however, they must necessarily represent accumulations of dead matter affording a passive nidus for the development of saprophytic fungi or other organisms capable of deriving nutrition from materials of animal origin, so that, if by any means the elements of these attain access to them, the normal course of the disease must be liable to be disturbed and its products come to contain fungal or other organised elements of extrinsic origin which do not represent causes but consequences of the primary morbid processes. In the case of the black variety of Mycetoma which was recorded in 1888 by Bassini* as having originated in Italy, the patient had sustained a wound in the foot in which the black fungiferous concretions subsequently were present, and in India the frequency with which the disease is associated with fungal elements, and the fact that fungoid concretions occur so much more frequently in the feet than in the hands, is probably connected with the facts that the natives of the country in conducting agricultural operations ordinarily go bare-foot, and that at particu-

* Un caso di micetoma ul piede o piede di Madura. Archivio per le scienze mediche, Vol. XII, p. 309.

lar times of year facultatively saprophytic fungi occur in the soil and its contents in very great abundance. It is possible that the changes produced in the degenerate products of the primary lesion by the access of fungal elements may be of an injurious character in so far as the ultimate outcome of the disease is concerned; but, as the evidence at present stands, there is no satisfactory ground for maintaining that the latter is primarily of fungal origin.

G. I. C. P. O.—No. 7 S. C.—28-11-95—225.

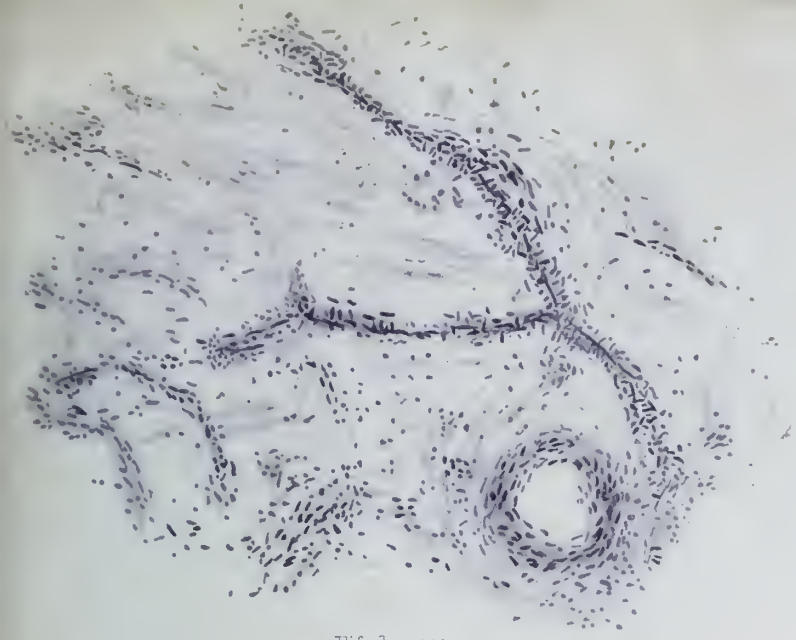


Fig 1 x160

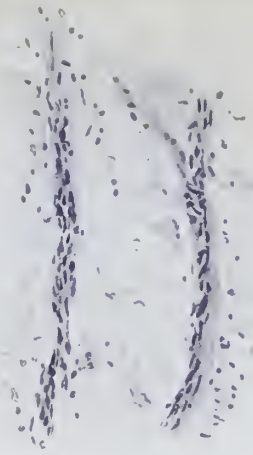
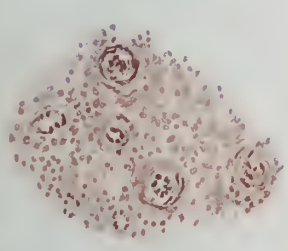


Fig 2 x160



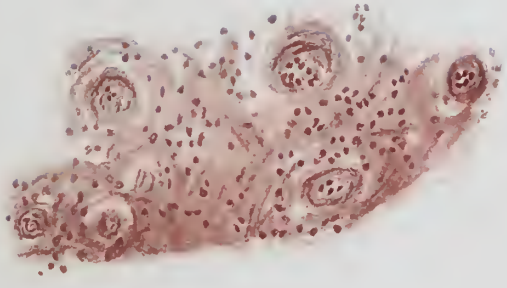


Fig 3 x160

Fig 4 x

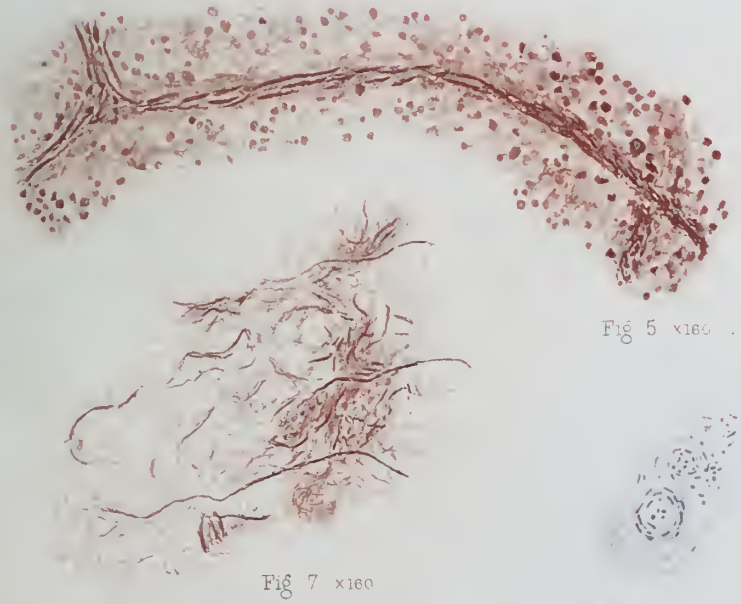


Fig 5 x160

Fig 7 x160

Fig 6 x160

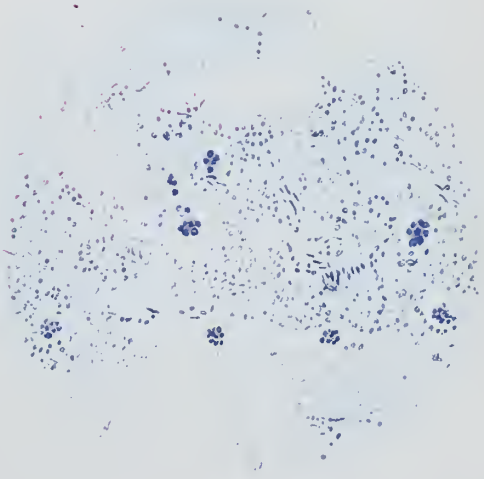


Fig 8 x160

MYCETOMA. CONDITION OF THE VESSELS IN THE INITIAL STAGES &c

Photo-etching

Survey of India Offices, Calcutta April 1891.

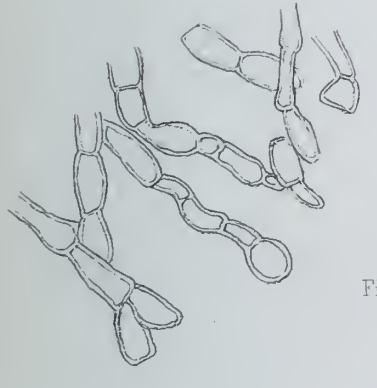
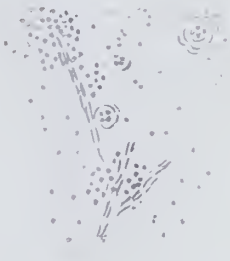


Fig. 2, × 830.



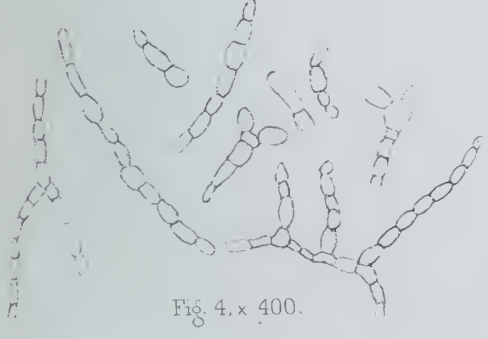


Fig. 4, × 400.

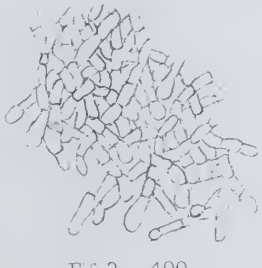
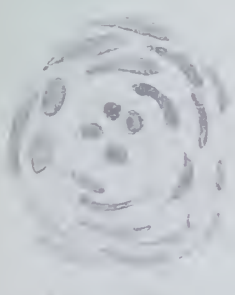
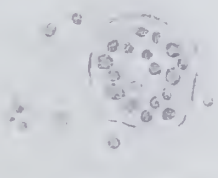


Fig. 3, × 400.





SCIENTIFIC MEMOIRS

BY

MEDICAL OFFICERS OF THE ARMY OF INDIA.

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SURGEON MAJOR-GENERAL J. CLEGHORN, M.D.,
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